Adsorption of Purified Cellulases on Cotton Fibers

by

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ABSTRACT

Commercial cellulases are a mixture of endoglucanases (EGs), exoglucanases (CBHs) and glucosidases. EGs and CBHs differ in their action on cellulose as a virtue of their structure but are adsorbed on cellulose to conduct hydrolysis. The nature of cellulase adsorption on cellulose has been explained by adsorption models such as Langmuir model in the past.

In the present investigation, Linear, Langmuir, Freundlich and combined Langmuir-Freundlich adsorption models were used to explain the adsorption of CBH I, II and EG II. CBH I, II and EG II were purified and adsorption of the purified enzymes on cotton fibers was collected. Statistical analysis was performed to determine the best fitting model.

It was found that more than one adsorption model can be used to explain the adsorption of CBH I, II and EG II on cotton fibers in the given range of enzyme concentration indicating that the substrate is not saturated by the enzyme. The temperature of adsorption was found to affect adsorption of CBH I, II and EG II on cotton fibers to a small extent. Adsorption of CBH I and II was found to be higher at 55 °C as compared to 25, 35 and 45 °C. Effect of temperature on adsorption of EG II on cotton fibers was more pronounced at higher concentrations of the enzyme.

INDEX WORDS: Cellulases, Endoglucanase, Exoglucanase, Cotton, Adsorption, Purification, Adsorption Model, Langmuir, Freundlich, Combined Langmuir-Freundlich,

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A Dissertation Submitted to the Graduate Faculty of the
University of Georgia in Partial Fulfillment of the
Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2003

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ACKNOWLEDGEMENTS

My experience at The University of Georgia was made memorable by the people that I interacted with here in Athens. I thank the university and the Department of Textiles Merchandising and Interiors for giving me the opportunity to pursue my graduate education.

My mentor, Dr. J. Nolan Etters has been a constant source of support and inspiration. I thank him for his timely advice, encouragement and his belief in my work. Dr. Etters has been a splendid advisor and I thank him for making my Ph.D. a great learning experience.

My committee members, Dr. Danny E. Akin, Dr. Patricia A. Annis, Dr. Lionel S. Carreira and Dr. Ian R. Hardin have offered their expert guidance and help in my research. I thank my committee members for their time and interest in my research. I am also grateful to the faculty and staff of the TMI department for helping me in every possible way and making my experience at UGA a pleasant one.

Dr. Lars Ljundahl and his research associate Dr. Irina Kataeva's help in my experimental work has made my research possible. I thank Dr. Ljundahl for his kindness to let me use the facilities at his laboratory. I also thank Dr.

Ljundahl and Dr. Kataeva for their expert guidance, suggestions and time to help me in my research.

I thank Dr. Avinash Manian and Dr. Ajoy Sarkar for their time to help me in writing my dissertation and make it a better document. I also thank Dr. Anupam Jaju who helped me in statistically analyzing my experimental data.

I would like to thank my fiancé, Chitra Thadhani for her constant support, patience and belief in me. I also thank Chitra’s parents for their help and encouragement.

Finally, I would like to thank my parents who have always encouraged and supported my education in every possible way. I thank my parents for their belief in my abilities and I hope that I will fulfill their dreams.

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INTRODUCTION

The use of enzymes in industrial textile processing dates back to 1857, when malt extract was used to remove starch-based size from printed textiles. Since then, commercial application of enzymes in textile processing has been slow [1]. Not until the 1960's did detergents make their appearance in the consumer market. Proteases were added to detergents to remove organic protein-based stains. Later, in the 1970's cellulases were added to detergent formulations to remove fibrils during fabric washing [2]. In the last decade, the use of enzymes in textile processing increased rapidly. Now, enzymes are being used in textile processes such as scouring, bleaching and finishing of textile substrates.

Enzymes are protein molecules that function as biological catalysts. After being adsorbed to a specific substrate, enzymes accelerate reactions at the surface of the substrate under environmentally benign conditions of temperature and pH. As a result, enzymatic processing leads to savings of energy and water. Being protein molecules, enzymes are biodegradable and, as catalysts, are required in small quantities [3, 4, 5]. Thus, the chemical oxygen demand (COD) and biological oxygen demand (BOD) of effluents from enzymatic treatments are low compared to chemical treatments [6, 7].

Among the different enzymes used in textile processing such as amylases, pectinases, cellulases, proteases and lipases, cellulases have been widely used in processing of cotton with great commercial success. Cellulases find applications such as additives in laundry detergents, defibrillation, bio-finishing of cotton fabrics and stone-washing of denim garments [8, 9, 10]. Commercially available cellulases are a mixture of three enzymes,

1. Endoglucanases (EG), also called "CMCase"
2. Exoglucanases (CBH), also called "Avicelase" or "Cellobiohydrolases"
3. Cellobiase [8,10]

Endoglucanases attack cellulose chains randomly along their lengths, acting on the amorphous regions of cellulose. Exoglucanases are thought to act on the crystalline regions of cellulose, attacking the polymer chain ends, generating cellobiose. Cellobiose is further hydrolyzed to glucose by cellobiase. For complete hydrolysis of cellulose to glucose, all three enzymes are required [8, 10].

Cellulases are produced by fungi and bacteria. *Trichoderma, Aspergillus, Penicillium* and *Fusarium* are common sources of fungal cellulases with *Trichoderma reesei* being the source of most commercially available cellulases [8]. Fungi may produce an array of endoglucanases and exoglucanases that may differ slightly in their action and topology. For example, cellulases from *Trichoderma reesei* contain two types of exoglucanases, CBH I and II, and six types of endoglucanases, EG I - VI. These different types of endoglucanases and exoglucanases differ in their structure and action on cellulose. It is difficult to break a large polymeric structure such as cellulose that is heterogeneous in its organization. Hence, multiple forms of endoglucanases and exoglucanases are required in order to bring about extensive hydrolysis of cellulose [7].

Recent studies on cellulase treatment of cotton fabrics are aimed at increasing its efficiency and effectiveness on cotton. Understanding adsorption characteristics of isolated endoglucanases and exoglucanases can throw light on the mechanism of their action on cotton fibers. Recycling and reuse of cellulase treatment baths can also be perfected with knowledge of adsorption characteristics of purified cellulases. Formulation of cellulase mixtures that can impart desired properties to cotton fabrics such as softness and removal of fuzz without substantial loss in weight and weakening of fabric can be possible with better understanding of the adsorption characteristics of purified cellulases.

Significance of Study

Cellulose is insoluble in water and hence the cellulose-cellulase system is heterogeneous system. Hydrolysis of cellulose by cellulase involves the following steps [11]:

1. Approach of enzyme to the substrate
2. Adsorption of enzyme on the substrate
3. Formation of an enzyme-substrate complex
4. Bond cleavage caused by cellulase and time course hydrolysis.

From the above steps listed involved in hydrolysis of cellulose by cellulase, it can be said that adsorption is a pre-requisite for cellulases to bring about cellulose hydrolysis. Adsorption of cellulases on the cellulose substrate is affected by a number of factors such as accessibility of the substrate, crystallinity and degree of polymerization [12, 13, 14, 15]. Cellulose substrates such as microcrystalline cellulose, pre-treated pulp and bagasse have been investigated for the action of complete cellulase systems and purified cellulases [16, 17, 18, 19, 20, 21, 22]. Microcrystalline cellulose is a commonly used substrate for such adsorption studies. Mathematical and empirical models have been developed to explain the kinetics of hydrolysis and understand the mechanism of hydrolysis. Since endoglucanases and exoglucanases differ in their action on microcrystalline cellulose, the composition of the enzyme mixture plays an important role in determining its action on cellulose [17].

Cotton differs in its crystallinity and accessibility from other cellulose substrates such as paper pulp, bagasse and pretreated wood chips. As a result, adsorption of purified cellulases on cotton is expected to be different from the other substrates. But studies involving the adsorption of endoglucanase and exoglucanase on cotton are very few [23, 24, 25]. Adsorption studies on cellulosic substrates including cotton have been conducted at low temperatures such as 3 °C and 25 °C in order to avoid the influence of hydrolysis due to cellulase action. In practice, enzymatic processing of cotton is conducted in the temperature range of 45 – 55 °C [26]. Hence, understanding adsorption characteristics of cellulase and its components on cotton at such practical conditions of treatment is important in order to make enzymatic processing of cotton fabrics more effective and efficient.

Adsorption models such as the Langmuir isotherm and Freundlich isotherm have been successfully used to describe the adsorption of dyes to the surface of textile fibers, the dyeing mechanism, as well as the kinetics of dyeing. Since the adsorption of cellulases on cotton is a surface phenomenon, adsorption isotherms may have the potential to explain the adsorption of cellulases on cotton fibers. Studying the adsorption characteristics of single enzymes may be important in optimizing the formulation and utilization of cellulase mixtures for treatment of cotton fabrics.

Purpose and Objectives

The purpose of this study was to determine the nature of adsorption of purified endoglucanases and exoglucanases on cotton fibers. Adsorption isotherms such as the Langmuir isotherm can provide a better understanding of the adsorption characteristics of endoglucanases and exoglucanases on cotton. Since adsorption isotherms describe the interaction of the enzyme with the substrate, isotherms can be useful in formulating mixtures of cellulases to impart specific properties to cotton fabrics. In order to conduct adsorption studies, endoglucanases and exoglucanases were purified from a commercial mixture of cellulases. Adsorption of increasing concentrations of purified endoglucanase and exoglucanases was assessed at different temperatures. Adsorption data was statistically analyzed performed to determine whether the data could be explained by different adsorption isotherms. Linear Regression analysis was conducted on the data using SAS® software.

The objective of this study was to determine whether the adsorption of endoglucanases and exoglucanases on cotton fibers can be explained using linear, Langmuir, Freundlich or Combined Langmuir-Freundlich adsorption isotherms.

Limitations of Study

1. Substrate hydrolysis during the course of adsorption of enzymes is not taken into account
2. Structural features of the substrate such as surface area and crystallinity are not studied. Any changes in the structural features due to hydrolysis are not taken into account when analyzing the results
3. Enzyme inhibition by substrate and by products is assumed to be minimal
4. Results and conclusions are valid over the range of temperatures and concentrations studied in this project which reflect the practical range used in enzymatic processing of cotton.
5. The isolated enzymes used in the present study may not be 100 percent pure.

CHAPTER 2

LITERATURE REVIEW

The processing of cellulose fibers can be categorized into dry processing and wet processing. In dry processing, bales of cotton fibers are opened and conditioned, cleaned by carding and combing, drawn and then spun into yarns. Yarns are then woven or knitted into fabrics. The aesthetic value of fabrics is increased by dyeing, finishing or printing before or after the fabric is converted into the end product [27]. In order to impart color and other desired properties such as softness, wrinkle-resistance and flame-retardance, the fabric should be absorbent. The presence of non-absorbent impurities such as natural waxes, pectin and added impurities such as lubricants and sizing agents makes it necessary to pre-treat the fabric. Pre-treatment processes include desizing, scouring, bleaching and mercerization. Pre-treatment processes require large amounts of water and energy. For example, a typical open width bleach range consists of desize, caustic and peroxide bleach stages. Each stage has a washing train that requires about 50-75 gallons of water per minute. The temperature of the water needed in such a range is at least 181 °F. The range consumes total of 16,450 pounds of steam per hour of which 10,350 pounds of steam is used by washing systems. The cost of processing textiles increases due to the increase in water consumption. For example, the amounts of water and energy used in a typical bleach range are listed in table 1 [28].

It has been estimated that careful optimization of bleach range configuration for types of fabrics processed, constant control of water and maintenance to avoid waste can lead to a savings of $20,000 per year in water costs (at the rate of $1.00/1000 gallons of water) and about $110,000 per year in energy costs (at the rate of $7.00/1000 pounds of oil-fired steam) [28]. Thus, reduction in water and energy consumption can account for a great reduction in the cost of textile wet processing.

Until the 1970s, production at low costs was the only consideration for a successful textile manufacturing facility. Pollution due to uncontrolled effluent release and inefficient use of resources were considered part of manufacturing cost. In recent years, as a result of increased level of environmental awareness of people and the government, companies unable to restrict levels of pollutants released into the environment are penalized [29]. As a result, the focus of textile manufacturers is to avoid environmental problems rather than find a solution after causing environmental problems.

Table 1. Water and Energy use in a Three-Stage Open-Width Bleach Range [28]

	Water (gal.hour) Incl. steam	Energy (lb. steam/hour)		
		Useful	Losses	Total
Saturators	550	300	400	700
Steamer and J-boxes	150	1150	150	1300
Washers				
Desize	3700)			
Caustic	3100)	8100	2250	10,350
Bleach	3150)			
Dry Cans	450	3600	500	4100
TOTAL	11,100	13,150	3300	16,450

Management strategies aimed at reducing water and energy costs include energy and water audits that help to identify water and energy costs for every process in the manufacturing unit. These costs can then be reduced by modifying the process. Textile mills are making efforts to reduce the level of pollutants in textile effluents by implementing the following strategies:

1. Treatment of effluents before release into the environment
2. Reuse of effluents after treatment
3. Recovery of chemicals and processing agents added
4. Substitution of chemicals that contribute to high COD and BOD of effluent

Enzymatic processing of textiles seems to be an attractive cleaner technology route to more energy efficient, less polluting and cheaper production processes.

Enzymes for Textile Applications

Enzymes have found applications in the following textile processes:

Desizing

Before weaving, warp yarns are strengthened in order to resist wear and tear during weaving by application of a size such as starch. After weaving, the size is removed to facilitate subsequent processing of the fabric. Alpha-amylases are used to remove starch-based size from yarns. Starch molecules are broken down to soluble compounds by α – amylase that are easily removed during washing [30]. Lipases are also added to desizing treatment baths in order to remove lubricants added to size formulations [31].

Scouring

Natural waxes embedded in the matrix of the primary cell wall of a cotton fiber protect it from the elements in nature. During scouring, hydrophobic impurities are removed along with some natural color to make cotton absorbent and white. Cellulases and pectinases are used in bioscouring of cotton fibers [32, 33, 34]. An alkaline pectinase is now commercially available for large scale enzymatic scouring. Bioscouring has been reported to reduce total water consumption by approximately 25%, retain fabric strength and lower weight loss [35]. Efforts are now being made to reuse cellulases and make bioscouring a continuous process rather than a batch process [36, 37, 38].

Bleaching

Traditionally, bleaching of cotton fabrics is done by hydrogen peroxide treatment at high temperature and pH. Hydrogen peroxide itself decomposes to water and oxygen and hence is environmentally safe, but the conditions required for peroxide bleaching are harsh to the environment. Hence a cheap and environmentally safe bleaching process is being investigated. Laccases, glucose-oxidases and xylanases (used in bleaching paper pulp) have been explored as bio-bleaching agents. Glucose-oxidases have been found to be the best alternative because of the following reasons:

i) Glucose-oxidases produce the highest improvement in whiteness of cotton fibers and

ii) Desizing effluents rich in glucose can be used in the bleaching process since glucose is required for the oxidation-reduction reaction [30, 39].

Bleach Clean-up

Residual peroxide from the bleaching process must be removed to ensure a good dyeing. In conventional processes, hydrogen peroxide is removed by two or more hot water rinses which make it a very energy and water intensive process.

Catalases decompose hydrogen peroxide to oxygen and water and their use in residual bleach clean-up needs only one hot water rinse at 80-95 °C. Not only is the residual hydrogen peroxide completely removed by catalase, but it also results in an overall process that is 6 to 8% cheaper than the conventional bleaching process [40, 41].

Denim Stone-washing

Denim stonewashing is a technique used to accelerate the fading and softening of jeans by washing denim garments along with pumice stones, patented by Francois Girbaud in 1978 [42].

The faded effect created by abrasion against pumice stones cannot be controlled and causes excessive damage to garments during washing. Also, a high content of suspended particles in the wash water leads to environmental problems. Since 1987, denim stonewashing is done using cellulases instead of pumice stones [43].

Bio-Finishing

The removal of surface fuzz (short fibrils and microfibrils) from cellulosic fibers with cellulase is termed as biofinishing or biopolishing. Enzymatic removal of surface fibrils results in smooth, clean-looking fabrics that retain their original color value. Other advantages of biofinishing are improved drapability, fashionable wash-down effects and improved moisture absorbency [43].

Effluent Treatment

Effluents from textile processes have to be treated in order to remove excessive pollutants, but mainly any colored matter present in the effluent. Living or dead organisms and selected microbes or enzymes may be used to treat effluents [44]. White rot fungi are found to degrade coloring matter very effectively in nature and hence enzymes from these fungi are being investigated to degrade colored impurities in textile effluents [45].

Current research focused on enzymatic processing of textiles is directed towards:

1. Making current enzymatic treatment methods efficient and effective [46]
2. Identifying new enzymes that can improve the properties of textiles [47].

For example, treatment with serine proteases on cross-linked cotton fabrics is being investigated. Serine proteases hydrolyze the amide bonds of cross-linked fabrics resulting in recovery of strength loss due to crosslinking. A short treatment with a low concentration of serine proteases at neutral pH and low temperature can result in efficient recovery of strength loss in amide crosslinked cotton fabrics [48]. Phosphorylation of cotton cellulose using hexokinase has been investigated as an alternative to chemical phosphorylation, which is a complicated process to introduce phosphorus groups in cellulose [49].

Advances in genetic engineering and enzyme technology are making it possible to develop enzymes with improved properties for textile applications. Biotechnology in textile industry will continue to offer solutions to make textile processing clean and eco-friendly.

Cellulases

Nature employs cellulases to break down cellulose produced by photosynthesis at a rate of at least 10^9 tons/year [50]. Most of the cellulose is broken down by cellulolytic organisms that use it as a source of carbon but degradation of cellulose by cellulases occurs through other means in nature. For example, trees shed leaves that are inefficient and unable to produce food through a process of senescence and leaf abscission. Near the end of the senescence process, abscission zones are created. Plant cells in this zone secrete pectinase and cellulase that degrade and reduce the strength of middle lamella and primary wall between cells, thus detaching leaves from the plant [51]. Plants also employ cellulases in the ripening of fruits.

An interest in this natural phenomenon of enzymatic hydrolysis of cellulose was generated during the World War II. After a brief period in a humid tropical climate, fungi ruined cotton gear, tents, uniforms and other military gear belonging to American units stationed in the South Pacific. Several laboratories within the Armed Services started investigating the nature of this “rotting”. Later, it was established that the fungus responsible for the destruction was a cellulose-destroying fungus [52].

Interest in cellulose hydrolysis was rejuvenated in the 1970’s when a global energy crisis emerged with the shortage of fossil fuels. Cellulose was looked upon as a renewable source of energy as it can be broken down to glucose, which on fermentation gives ethanol – an alternative source of fuel. Mineral acids were used for the break down of cellulose, but the process is difficult to control, needs acid resistant containers and is not environmental friendly. Cellulases were employed to hydrolyze cellulose to glucose instead of acids [52]. Recent applications of cellulases include the selective modification of cellulosic materials and surface treatment without affecting mechanical properties of the substrate. Cellulase hydrolysis and action of cellulases on cotton fibers has been studied by researchers in the past [53] [54]. In these studies the effect of different factors affecting cellulose hydrolysis such as agitation and pretreatment of cotton has been studied [55, 56, 57]. The effect of enzymatic treatment on the hand properties, dyeability and tensile strength of cotton has also been studied in the past [58, 59, 60, 61, 62]. Knowledge of the properties of the substrate and enzymes makes it easy to understand the action of cellulases on cotton.

Structure of endoglucanases and exoglucanases

In 1986, Van Tilbeurgh[50] showed that exoglucanase I (CBH I) can be dissected into two functional domains by limited proteolysis. Later, other enzymes such as CBH II were also found to have a similar arrangement. A general architecture of cellulases was then proposed based on these studies. A cellulase molecule can be divided into the following [50, 63]:

1. catalytic domain responsible for hydrolysis of cellulose
2. cellulose binding domain that promotes adsorption onto the insoluble cellulose
3. linker peptide that joins the two domains together

Structural investigations using small angle X-ray scattering (SAXS) later confirmed the two domain architecture of cellulases [50].

Catalytic Domains:

Based on the amino acid sequence, catalytic domains of cellulases can be grouped into 6 families, A-F. Each family of catalytic domain is characterized by a conserved fold and different folds are expected in different families. This implies that enzymes belonging to the same family will show a similarity in folding pattern resulting in an overall conservation of active site topology. The endo- or exo mode of action of cellulases is determined by the specific details of 3D structure. Endo and exo cellulases in the same family have a similar global 3D fold but differing substrate specificity due to minor details of the structure. For example: CBH II from *T. reesei* that belongs to Family B has a β/α barrel structure with the active site having a tunnel shape. Endoglucanases of the same family were found to have similar overall fold but their active site was found to open into a cleft allowing random binding of the cellulose chains [50, 64].

Cellulose Binding Domain (CBD):

CBDs can be grouped into five distinct families (I – V) based on amino acid sequence similarities with their catalytic domain. Most CBD’s are structurally well defined domains linked to either the N- or C-terminus of the catalytic domains. CBDs binding to crystalline cellulose may differ in size and topology but show a similar binding interaction [65].

The primary function of CBDs is believed to be attaching the cellulase on the surface of the substrate, but CBDs can take a more active part in cellulose degradation than the mere anchoring of cellulases to their substrate. CBDs are found to increase the concentration of the enzyme on the substrate and hence it may imply that the presence of CBDs increases the enzymatic activity of cellulases. Since catalytic domains are responsible for hydrolysis, the presence of CBDs may have little or no influence on activity of cellulases [66]. CBDs might help in loosening the individual cellulose chains from the cellulose surface prior to its actual hydrolysis. CBDs can penetrate the fibers at surface discontinuities thereby releasing noncovalently attached fragments and uncovering new cellulose chain ends [66].

The best-characterized type of CBD is type 2. CBDs of type 2 have been found exclusively among fungal cellulases. The domain is shaped like a wedge whose dimensions are approximately 30X18X10 °A. One side of the wedge is hydrophilic, and the other is hydrophobic. The hydrophilic side which presumably makes contact with the surface of the substrate contains three tyrosines. It has been proposed that the wedge-like shape of fungal CBDs not only promotes binding to the substrate, but also helps to peel off the cellulose chains from the top layer of cellulose microfibrils [66].

Linker Peptide:

The catalytic and cellulose binding domains in exoglucanases and endoglucanases are connected by a linker peptide. Since the cellulose binding domain may get attached to the cellulose substrate, the linker peptide provides a dynamic attachment of the catalytic domain on the surface of the substrate. The interdomain region may vary in length in different forms of endoglucanases and exoglucanases, thus affecting their enzymatic activity. The linker peptide is sensitive to proteolytic digestion and hence some culture solutions may contain enzyme forms both with and without CBDs [65].

Exoglucanases:

CBH I make up 60% and CBH II 20% of the total cellulolytic protein in cellulases from *Trichoderma reesei*, accounting for most of its cellulolytic activity. These two enzymes can achieve complete, although slow, solubilization of cellulose crystals even without the help of endoglucanase [63].

Exoglucanases have tunnel shaped active sites. Active sites are located in tunnels enclosed by two or four long loops [67]. The active sites of exoglucanases (CBH I and CBH II) are designed to bind a single glucose chain in a defined orientation [68]. For both enzymes, cellobiose is the major reaction product released from the non-reducing end of the chain by CBH II and from the reducing end of the chain by CBH I. It is thought that a single cellulose chain is fed into these tunnels from one end, followed by their threading through the entire length of the tunnel and subsequent bond cleavage and release of the cellobiose product from the far end of the tunnel. The remaining chain is still bound to the enzyme through interactions at a number of sub-sites. This creates the conditions for a processive mode of action in which several consecutive hydrolytic events occur without dissociation of the enzyme and the substrate.

CBH II has a shorter tunnel and hence falls off the substrate and reinitiates hydrolysis at an adjacent chain end.

CBH I which has a much longer tunnel would bind more tightly to the remaining glucan chain and is less likely to dissociate after each hydrolytic event. Therefore once bound to an available chain end, CBH I would continue hydrolyzing to the end. Cellobiohydrolases similar to CBHII, which have shorter active-site tunnels, may exhibit increasing degrees of endoglucanase type activity due to fewer loops with different lengths and/or mobilities.

Endoglucanases:

Endoglucanases preferentially attack internal glycosidic linkages of substrates such as acid swollen cellulose and soluble glucan, besides modified substrates such as CMC. Endoglucanases with open active sites can bind to and act in the middle of the glucan chains. The sequences corresponding to the active-site loops of exoglucanases are not present in homologous endoglucanases [69].

Structure of cotton fiber

A cotton fiber is a single cell fiber growing from a modified epidermal cell of cotton seed. Cotton is made of cellulosic and noncellulosic components. Table 2 lists the percentage composition of the cellulosic and noncellulosic components present in a typical cotton fiber [70]. Cellulose is present as fibrils organized in different layers whereas the noncellulosic

Table 2. Chemical composition of cotton fibers [70]

	Percent (dry basis)		
Constituents	Typical	Low	High
Cellulose	94.0	88.0	96.0
Protein	1.3	1.1	1.9
Pectic substances	0.9	0.7	1.2
Ash	1.2	0.7	1.6
Wax	0.6	0.4	1.0
Malic, citric and other organic acids	0.8	0.5	1.0
Total sugars	0.3		
Pigment	Trace		
Other	0.9		

substances such as pectin, fats and waxes are largely confined to the outer layers of the fiber. Figure 1 represents the fiber structure of cotton that is divided into the following layers [71]

1. cuticle
2. primary wall
3. Secondary wall

Cuticle: The cuticle of cotton contains waxes and pectin in about equal amounts and is so intimately bound to the primary wall that it may not be considered as a separate layer.

Primary wall: The primary wall is less than half a micrometer (µm) in thickness. The primary wall contains the bulk of the noncellulosic constituents of the cotton fiber. The cellulosic portion in this layer is a network of microfibrils interlaced in a fabric in which the general system of orientation is axial on the outer surface and transverse on the inner surface. Also, the cellulose fibrils of the inner surface of the primary wall are finer than those of the outer face [72].

Secondary wall: The secondary wall of cotton fiber is entirely cellulose. The secondary wall can be divided into the S-1 layer, S-2 layer and the lumen wall or S-3 layer. The S-1 layer is the very first layer of cellulose deposited after the cell grows to its full length. The S-1 layer is coarse and the fibrils arranged in a banded structure of alternate close and

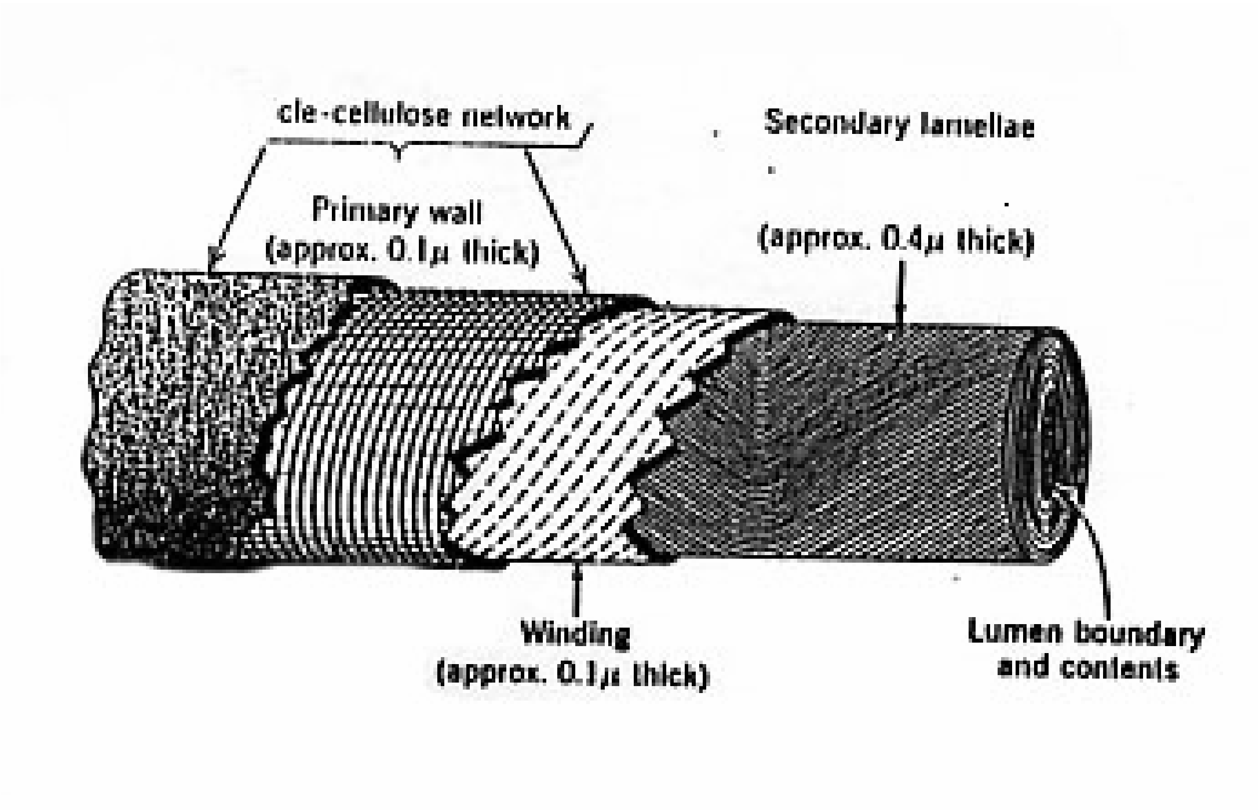

Figure 1. Structure of a Cotton Fiber [71]

open packing. The bands appear to spiral about the fiber axis at an angle of 45 degrees. The fibrils in the S-1 layer periodically reverse in direction and so a system of left and right (S and Z) spirals is set up along the length of the cotton fiber.

The S-2 layer is the main body of the fiber and can be as much as 4 to 5 µm in thickness. The layers in this part of the secondary wall can be separated fairly easily when swollen in a solvent for cellulose. The cellulose of the S-2 layer appears to occur in compact layers of closely aligned microfibrils which lie almost parallel to each other and at a slight angle to the fiber axis. The direction of the spiral in the S2 layer is opposite to that of the spiral in the S1 layer.

The lumen wall or the S-3 layer may or may not be present and is found to be more resistant to reagents [73]. The hollow space inside the collapsed fiber is known as the lumen.

Adsorption models

Adsorption isotherms traditionally used in adsorption of gases and liquids on surfaces has been used to model cellulase adsorption. The models most commonly used in the cellulose-cellulase system are [74, 75]:

a. Linear Adsorption Isotherm
b. Langmuir Adsorption Isotherm

c. Freundlich Adsorption Isotherm
d. Combined Langmuir-Freundlich Adsorption Isotherm

In most adsorption studies, microcrystalline cellulose has been used as the substrate. Adsorption studies have also been conducted on natural substrates such as bagasse, newspaper pulp and treated wood chips [76, 77, 78]. In some adsorption studies cellulase has been considered as a multicomponent adsorbate whereas in other studies purified cellulases have been used.

A. Linear Adsorption Isotherm

The linear adsorption isotherm is also known as the Nernst's adsorption isotherm. The isotherm can be represented by the equation as follows [79]:

$$[A] = K_d[E] \qquad \text{Equation 1}$$

where, [A] = amount of enzyme adsorbed on cotton fibers

[E] = amount of enzyme present in the solution

K_d = constant given by the slope

In this model, the amount of enzyme adsorbed by the substrate is proportional to the concentration of enzyme initially present in the solution.

B. Langmuir Adsorption Isotherm

The Langmuir adsorption model is based on two assumptions

1. The substrate is composed of uniform binding sites and,
2. There is no interaction between the adsorbing molecules.

The Langmuir model assumes that a monolayer of adsorbents is formed and that the free energy of adsorption remains constant.

Peiterson,et.al.[74], were the first to suggest that cellulase adsorption on microcrystalline cellulose can be explained by the Langmuir isotherm. The authors studied adsorption of a mixture of cellulase (crude cellulase) on microcrystalline cellulose. Microcrystalline cellulose was treated in an aqueous bath. The mixture of enzymes and cellulose was then centrifuged and the protein content in the supernatant was determined. The amount of adsorbed protein can be obtained by the following equation:

$$P_{ads} = [P]\frac{K_p \cdot P_{ads,m}}{(1 + K_p.P)} \qquad \text{Equation 2}$$

where,

P = protein concentration in supernatant (mg/ml)

P_{ads} = adsorbed protein (mg of protein/mg of cellulose)

$P_{ads,m}$ = maximum amount of protein adsorbed (mg of protein/mg of cellulose)

K_p = constant $(mg/ml)^{-1}$

Kim et.al.[80-85] studied the adsorption of individual endoglucanase and exoglucanase components on microcrystalline cellulose. The equation that the authors used to describe the adsorption of various types of endoglucanase and exoglucanase on microcrystalline cellulose is another form of the Langmuir

Isotherm. The experimental data obtained to fit the following equation,

$$[A] = \frac{[A_{max}]\ K_{ad}\ [E]}{1 + K_{ad}[E]} \qquad \text{Equation 3}$$

where,

A_{max} = maximum amount of enzyme adsorbed per unit weight of cellulose

K_{ad} = adsorption equilibrium constant

[E] = concentration of enzyme in liquid phase at adsorption equilibrium

The term K_{ad} was used by the authors as a measure of the affinity of cellulase to cellulose and to calculate thermodynamic parameters of the adsorption process such as change in heat of enthalpy (ΔH_a), change in Gibbs free energy (ΔG_a) and change in entropy (ΔS_a) [81] [84] [85].

Another form of Langmuir adsorption isotherm was suggested by Medve et.al [75]. The authors attempted to fit the adsorption data they obtained in their experiments to different adsorption isotherms that were used to explain adsorption of other proteins. The Langmuir adsorption model used by Medve et.al, [75] is given as follows:

$$B = \frac{n[F]}{K_d + [F]} \qquad \text{Equation 4}$$

where,

B = amount of the bound enzyme (μmol/g of substrate)

F = concentration of free enzyme in solution (μmol/L)

n = number of binding sites per gram of substrate (μmol/g)

K_d = dissociation constant of the adsorbent-adsorbate complex (μmol/L)

The authors characterized the affinity of the adsorbate (cellulase) to the adsorbent (cellulose) by a term called the distribution coefficient - α. Alpha is given by the initial slope of the Langmuir isotherm, which is given as

$$\alpha = \frac{n}{K_d} \qquad \text{Equation 5}$$

The authors used a modified Langmuir adsorption model that assumed the presence of more than one kind of binding site written as follows

$$B = \frac{n_1[F]}{K_{d1} + [F]} + \frac{n_2[F]}{K_{d2} + [F]} \qquad \text{Equation 6}$$

n_1 and n_2 = (μmol/g) number of adsorption sites/g of substrate

K_{d1} and K_{d2} = (μmol/L) dissociation constant for the two sites

Distribution coefficient of the mixture of adsorbent is given as the sum of the distribution coefficient for the two sites,

$$\alpha = \frac{n_1}{K_{d1}} + \frac{n_2}{K_{d2}} \qquad \text{Equation 7}$$

and the total number of sites is given as $n = n_1 + n_2$

C. Freundlich Adsorption Isotherm

In an attempt to account for structural heterogeneity of cellulose substrates, the Freundlich isotherm was used to describe adsorption of cellulases [75]. Freundlich isotherm has been used to describe adsorption of α-amylase to starch granules.

The Freundlich equation can be derived from a Langmuir equation by assuming a certain distribution function for sites having different free energy values for the adsorption process and assuming Langmuir adsorption at each type of site [75]. Freundlich isotherm for adsorption to heterogeneous surfaces is given as follows:

$$B = K[F]^{1/m} \qquad \text{Equation 8}$$

where,

K = Freundlich Equilibrium constant

m = power term of the Freundlich Isotherm ($m>1$)

n = (μmol/g) number of binding sites/g of substrate

m and K are empirical parameters.

D. Combined Langmuir-Freundlich Model

The combined Langmuir-Freundlich isotherm used to describe adsorption to a heterogeneous surface is given as follows:

$$B = \frac{n[F]^{1/m}}{K + [F]^{1/m}} \qquad \text{Equation 9}$$

where,

m = empirical constant

K = apparent adsorption constant.

Equation 9 can describe experimental data for adsorption of cellulases as accurately as the 2-site Langmuir model with fewer parameters, but interpretation of the derived parameters is difficult [86].

Apart from the models described, other models have been used to describe cellulase adsorption on cellulose [75]. The Langmuir isotherm however is by far the most widely used isotherm to describe adsorption of cellulases to cellulose.

Adsorption of Exoglucanases

From the adsorption equilibrium constants, Kim et.al, [81] suggested that CBH I exhibits a greater affinity to cellulose compared to CBH II.

Kim et.al, [74][81] found that a mixture of CBH I and CBH II in the weight ratio of 1:1 adsorbed more spontaneously than either CBH I or CBH II individually. The authors suggested that

CBH I and CBH II partially form a CBH I-II complex which has a higher affinity for cellulose than the individual components. The Langmuir adsorption isotherm was found adequate to explain the adsorption data. On the other hand, Medve et.al., [75] [86] reported that the combined Langmuir-Freundlich adsorption or the 2-site Langmuir adsorption model described the adsorption of CBH I and II, together from a mixture, with a greater correlation than what was obtained from the Langmuir isotherm.

Adsorption of Endoglucanases

Adsorption of mixtures of endoglucanases as well as the individual endoglucanases such as EG I and EG II has been investigated by many researchers [59] [82] [83] [84] [85] [86]. Beldman et.al, [87] identified and studied the adsorption of six endoglucanases as EG I-VI. Based on values of the initial slope of adsorption isotherms plotted, EG I, III and V adsorbed more strongly than EG II, IV and VI. Results suggested that enzymes that adsorbed strongly are not necessarily less random in hydrolytic activity than the weakly adsorbed enzymes. Contradictory to this classification of endoglucanases reported, Kim, et.al, [82] found that EG I and II have higher affinity than EG III and IV based on their adsorption equilibrium constants. Kim et.al.,[82] also suggested that the endoglucanases with high affinity to cellulose bind at sites where the crystalline structure of the substrate is disturbed. It was suggested that endoglucanases induced defibrillation of the crystallites and opened new sites for the action of exoglucanases. Adsorption of crude and different types of endoglucanases followed Langmuir adsorption [83, 84, 85].

Adsorption of Mixtures of Endoglucanases and Exoglucanases

A mixture of crude endoglucanases and exoglucanases in weight ratio of 1:1 was found to show synergistic action on cellulose hydrolysis. An optimal ratio of endoglucanase and exoglucanase should be maintained during the course of hydrolysis, for maximum hydrolytic action. However, it has been suggested that the ratio may not be maintained, for example, because of a decrease in number of sites for endoglucanases and increase in number of sites for exoglucanases and individual nature of adsorption of endoglucanases and exoglucanases [85, 88].

The composition of a mixture of various endoglucanases and exoglucanases would affect the adsorption behavior due to differences in adsorption characteristics of types of endoglucanases and exoglucanases. Mixtures of individual types of endoglucanase or exoglucanases in different combinations have been studied. Some components of endoglucanase and exoglucanase show synergism in hydrolytic action while others do not show any synergism. For example, CBH II shows synergistic action when present in weight ratio of 1:1 with EG I or EG II [82, 83, 84]. A mixture of EG I and CBH II hydrolyzes microcrystalline cellulose to a greater extent than EG I or CBH II individually. CBH II showed synergistic action in the presence of EG II but in presence of EG I, CBH II showed preferential adsorption on microcrystalline cellulose. CBH II was found to be inactive on microcrystalline cellulose when used alone.

Summary

Most of the studies described in the literature survey are conducted on cellulose substrates such as microcrystalline cellulose. Since there may be differences in the properties of microcrystalline cellulose and cotton, the adsorption characteristics of cellulases on cotton fibers may be different. In addition, the temperatures at which most studies described in the survey of literature were performed are not a representation of the temperature of textile wet processing conditions. Hence it is important to study the adsorption characteristics of purified cellulases on cotton under practical conditions of temperature and concentration.   A study of the adsorption of purified cellulases will be helpful in understanding the action of cellulases on cotton and also in developing cellulase formulations that are more efficient.

Chapter 3

MATERIALS AND METHODS

Materials

Substrate

Raw cotton fibers (Texas Grade 52, Low Middling Light Spot Cotton) were obtained from Avondale Mills, Monroe, GA. The fibers were then cleaned and used in this study.

Enzymes

Celluclast® a commercial mixture of endoglucanases and exoglucanases produced from Trichoderma reesei, a fungus mainly used for commercial production of cellulases, was obtained from Novozymes NA, Franklinton, North Carolina. Endoglucanases and exoglucanases required for the adsorption experiments were purified from this commercial mixture using Fast Protein Liquid Chromatography (FPLC).

Spectrophotometer

A Spectronic Genesys 2 UV-VIS spectrophotometer was used for single spectrophotometric measurements. For large numbers of spectrophotometric measurements, a microplate spectrophotometer, 340 ATTc, was used.

Reagents

The following reagents were used in the purification of enzymes:

1. Ammonium sulfate, Reagent grade supplied by J.T.Baker
2. Tris(hydroxymethyl)aminomethane also known as Trizma Base or Tris, Reagent grade supplied by Sigma
3. NaCl, Reagent grade supplied by J.T.Baker
4. Hydrochloric acid, Reagent grade supplied by J.T. Baker

The following reagents were used in the adsorption experiments:

1. Sodium acetate, Reagent grade supplied by J.T. Baker
2. Acetic acid, Reagent grade supplied by J.T. Baker
3. Cellobiose, Reagent grade supplied by J.T. Baker
4. 2,4 – dinitro salicylic acid (DNS), Reagent grade supplied by Sigma

Methods

Substrate Purification

Impurities such as vegetable matter, seed fragments and very short fibers were removed by passing the fibers through a Shirley Analyzer. Cotton fibers were then scoured at 80 °C for 60 minutes. The material to liquor ratio used was 1:40. Scouring solution was prepared using the following ingredients:

- Sodium Carbonate: 5 gpl
- Wetting Agent: 1 gpl (Clavodene MCM-2)

- Chelating Agent: 1 gpl (Barapon C-108)

The scouring solution was heated to 60 °C and the cotton fibers were introduced into the bath. The bath was stirred with a glass rod intermittently. After 60 minutes the cotton fibers were collected and washed with hot and cold water. The fibers were then washed with deionised water until the pH of wash water pH was 7. The fibers were then air dried and stored in a standard atmosphere at 65% R.H. and 21±1 °C.

Enzyme Purification

Purification of Enzyme:

Endoglucanases and exoglucanases were purified from a commercial cellulase mixture, Cellulclast, using Fast Protein Liquid Chromatography (FPLC) system [89] [90]. The purification of the endoglucanases and exoglucanases involved the following steps:

1. Sample Preparation
2. Separation of enzyme components on DEAE Sepharose CL-6B column
3. Separation of enzyme fractions on SP-Sepharose column
4. Separation of enzyme fractions on Mono S column HR 10/10

Different columns were packed for the separation of endoglucanases and exoglucanases using the FPLC system. The packing resins to be packed in columns for the separation of enzymes were obtained from Amersham Pharmacia Biotech, Piscataway, NJ. The properties of the packing resins in different columns are listed below:

1. HiPrep 26/10 Desalting column packed with Sephadex G-25 Fine. Sephadex G-25 Fine is commercially available bead-formed gel prepared by cross-linking dextran with epichlorohydrin. This column available as a packed column was obtained from Amersham Pharmacia Biotech, Piscataway, NJ, USA.
2. DEAE Sepharose CL-6B column is packed with DEAE Sepharose CL-6B, a commercially available weak anion exchanger with diethylaminoethyl as the ion exchange group.
3. SP Sepharose XL column is packed with SP Sepharose XL, a commercially available strong anion exchanger based on a highly cross-linked, bead formed 6% agarose matrix. Dextran chains are covalently coupled to the agarose matrix and sulphopropyl ion exchange groups are attached to this dextran through chemically stable ether bonds.
4. Mono S HR 10/10 is a commercially available strong cation exchange column based on a beaded hydrophilic resin with a particle size of 10 µm. The charged group on the gel is methyl sulphonate. The capacity of the gel is 0.14-0.18 mmol/ml and substances with molecular weights up to 10^7 can be successfully separated. The protein capacity is in the

range of 20-50 mg/ml gel. Mono S HR 10/10 is a column readily available with Amersham Pharmacia Biotech, Piscataway, NJ.

1. Sample Preparation: Celluclast was prepared prior to loading on the DEAE-Sepharose column for separation of the individual endoglucanases and exoglucanases. In this process, diluents in Cellulast mixture such as sorbitol and sodium chloride and preservatives such as potassium sorbate were separated from the proteins. The proteins in the commercial cellulase mixture solution were precipitated with 75% by weight of ammonium sulfate. After precipitation, the solution was centrifuged and the precipitate was collected by decanting the supernatant salt solution. The precipitate was dissolved in a minimum volume of tris-HCl buffer. In the initial trials three different methods were employed to remove any residual ammonium sulfate from the solution – dialysis, ultrafiltration and desalting column.

The dialysis bags are made of modified cellulose and hence are easily hydrolyzed by cellulase solution resulting in the breaking of dialysis bags. Repeated trials using dialysis bags indicated that short periods of dialysis were effective and did not result in breaking of the dialysis bags. Traces of ammonium sulfate from the enzyme solution could also be removed by ultrafiltraion using a polyethersulfone membrane, PM30 manufactured by Millipore, Billerica, Massachussetts. The enzyme solution was placed in the ultrafiltration cell and nitrogen gas passed through the ultrafiltration cell to prevent denaturation of the protein. The salt solution was filtered through the membrane under vacuum and discarded after testing for the presence of protein by the Bradford method [ ]. During ultrafiltration, the protein solution was stirred continuously to prevent clogging of the ultrafiltration membrane. Ultrafiltration of highly concentrated protein solutions was difficult since the membrane got clogged over time and had to be removed and washed periodically. Desalting of the protein solution was tried using a desalting column HiPrep 26/30. The gel filtration column separates the components of the solution into based on their molecular weights. The high molecular weight compounds such as the protein molecules are excluded from the gel and so eluted first whereas the low molecular weight substances such as ammonium sulfate enter the pores and are thus eluted later. The desalting column was found to be very efficient with protein solutions of low concentrations. The use of desalting columns did not require precipitation of protein from commercial mixture using ammonium sulfate.

Desalting of Celluclast was repeated using small volumes each time until sufficient quantities of desalted protein solution was collected.

2. Separation of Enzyme Components on DEAE- Sepharose CL-6B Column:

DEAE Sepharose 20 mM Tris-HCl buffer (Buffer A) at pH 7.5 was used as a starting buffer. The buffer was used to load the column with the desalted protein solution. The flow rate of the starting buffer was maintained at 5 ml/min. The eluting buffer used was 20 mM Tris-HCl solution at pH 7.5 with 1 M NaCl. A salt gradient of 0-100% salt was provided by the 1 M NaCl present in the eluting buffer.

When the mixture of enzymes was injected, CBH I was adsorbed onto the column and later eluted by the elution buffer. CBH II and EG II did not adsorb to the DEAE Sepharose column and were eluted with the starting buffer A [89, 90]. CBH I was then concentrated by precipitation with ammonium sulfate and dialysis. The purity of the enzyme was later checked by SDS Page Gel Electrophoresis.

3. Separation of enzyme fractions on SP-Sepharose XL column

The unadsorbed fraction of the enzyme mixture containing CBH II and EG II was concentrated by precipitation with 75% ammonium sulfate followed by desalting by using the Fast Desalting Column (Pharmacia). The concentrated fraction was then injected into a SP-Sepharose column using a 10 mM sodium acetate starting buffer at pH 4.0(Buffer A). EG II did not adsorb on the SP-Sepharose XL column and was eluted along with the starting buffer [90]. The eluting buffer used was 10mM sodium acetate buffer with 0.5 M NaCl. The adsorbed CBH II was eluted with the elution buffer and concentrated by precipitation and dialysis. The fraction unadsorbed in SP-Sepharose contained more than one enzyme and hence had to be purified to homogeneity.

4. Separation of enzyme fractions on Mono S HR 10/10 column

The unadsorbed fraction was then injected into a Mono S column HR 10/10 using a starting buffer of 10 mM sodium acetate at pH 3.0. The eluting buffer used was 10 mM sodium acetate and 1M NaCl. All the protein was adsorbed on the column and then eluted with low salt whereas the other fraction was eluted at high salt concentration. EG II fraction was then checked for purity by preparing a SDS PAGE Gel.

At the end of the enzyme purification process, pure fractions of CBH I, CBH II and EG II were obtained.

Measurement of Purity of Enzymes:

The purity of separated endoglucanases and exoglucanases was measured using Sodium Dodecyl Sulphonate- Polyacrylamide Gel Electrophoresis (SDS-PAGE) [91]. Electrophoresis is the process of moving charged molecules in solution by applying an electric field across the mixture. The migration of the molecules to a charged pole is dependent on their charge, shape and size [92, 93].

Procedure:

1. Acrylamide Gel Preparation: The polyacrylamide matrix used in SDS-PAGE electrophoresis is divided into two different layers, the lower layer or the separating or resolving gel (12% acrylamide) and the upper layer or the stacking gel (4% acrylamide). The compositions of these layers are given in Table 3. The separating gel is responsible for separating the polypeptides by size and the stacking gel causes the samples to be compressed or stacked in order to have thin bands. Ammonium persulfate (PSA) is used as the indicator peroxide and N, N, N’, N’- tetramethylethylenediamine (TEMED) is used as a catalyst to polymerize the arcylamide solution. The preparation of the acrylamide gel is a standard procedure and the details were obtained from the electrophoresis handbook provided by Amersham Biochem [94].

Enzyme denaturization: Enzyme solutions of 5 and 10 µg/µl of CBH I, II and EG II were prepared. The protein solutions were boiled with an equal amount of loading buffer. The loading buffer contained tris buffer (0.25 M, pH 6.8), 20% SDS, mercaptoethanol, glycerol and bromophenol blue. SDS, which is an anionic detergent, denatures proteins by wrapping the

Table 3. Composition of the separating and stacking gels in SDS PAGE Gel

	Separating	**Stacking**
Acrylamide	2.09 mL	0.33 mL
SDS (20%)	25 µL	12.5 µL
Tris	1.5 µL	1.25 µL
Temed	2.5 µL	2.5 µL
Water	1.8 mL	0.765 mL
PSA	125 µL	125 µL

(SDS stands for Sodium dodecyl sulfonate, Tris stands for tris(hydroxyl)aminomethane, temed stands for N, N, N′, N′-tetramethylethylenediamine and PSA stands for ammonium persulfate) hydrophobic tail around the polypeptide backbone. SDS confers a net negative charge to the polypeptide in proportion to its length.

2. Electrophoresis: The denatured enzyme solutions were then poured into the wells of the stacking gel. A low molecular weight marker was added to one of the wells on the stacking gel. The marker acts as a reference and may be used to estimate the molecular weight of the enzyme. The gel was then placed in an electrophoresis tank and the buffer was slowly poured into the tank. A continuous buffer system (0.25 M tris, 1.92 M glycine and 0.1% SDS) was used in this experiment. The electrodes were connected and a potential difference of 160 V was applied across the gel for 50 minutes. At the end of 50 minutes, the gel was removed from the buffer tank.
3. Staining and Destaining of the gel: The gel was removed from the stand and placed in 50 ml of staining solution for 2 hours. The staining solution was prepared by mixing 0.2% Coomassie Brilliant Blue R-250 dye, 45% methanol and 10% acetic acid. The stained gel was then placed in a container with destaining solution. The destaining solution was prepared by mixing 100 ml of glacial acetic acid, 200 ml of ethanol and 700 ml of water (v/v). Destaining was conducted overnight with shaking provided by placing the container on a shaker operated at 80 rpm. The gel was then removed from the

destaining solution, washed with deionized water, dried and photographed [94].

Enzyme Adsorption Procedure

Enzyme solutions for the following concentrations (mg/ml) were prepared from the concentrated solutions of pure enzymes;

CBH I: 0.493, 0.737, 0.976, 1.159, 1.412, 1.827, 2.472
CBH II: 0.477, 0.715, 0.929, 1.315, 1.335, 1.436, 1.774
EG II: 0.362, 0.690, 0.997, 1.140, 1.556, 1.857, 2.069

Adsorption of enzymes was conducted in clean test tubes. One milliliter of enzyme solution was taken in a 15 ml test tube. The test tubes were placed in a water shaker bath operated at 50 rpm and heated to the pre-determined temperature. After the enzyme solution reached the set temperature, 0.1 grams of cotton fibers were added to each test tube. The test tubes were incubated for 60 minutes after which the fibers were removed from the bath. The enzyme solution was centrifuged at 14000 rpm for one minute to remove any suspended particles. The supernatant was transferred to clean Eppendorf tubes and stored at 3 °C until the protein was measured.

Analytical Procedures

Determination of Protein in Solution

The amount of protein present in solutions was measured using two methods:

1. Bradford method using Coomassie Brilliant Blue G-250 was used for quick measurements [95].
2. Bicinchoninic acid method (BCA method) was used for large number of measurements [96].

Bradford Method

Principle:

The anionic form of Coomassie Brilliant Blue G-250 present in acidic conditions forms a complex with protein. The binding of the dye to the protein results in an absorbance shift from 465 to 595 nm. The absorbance of the dye-protein complex is proportional to the protein concentration. Bovine Serum Albumin solution is used as the standard for calculating the amount of protein present in solutions.

Spectrophotometric Measurement of Color:

Ten microliters of supernatant solution were added to 1 ml of Bradford reagent. After 15 minutes, the absorbance was measured at 595 nm. A calibration curve plotted for a range of Bovine Serum Albumin concentrations was used to determine the protein concentration.

BCA Method

Principle:

Cupric ions (Cu^{+2}) are reduced by protein in an alkaline medium to cuprous ions (Cu^{+1}). The cuprous ions (Cu^{+1}) can be selectively detected by a highly sensitive detection agent, bicinchoninic acid (BCA). Two molecules of BCA react with one cuprous ion (Cu^{+1}) and form a purple-colored complex that exhibits a strong absorbance at 562 nm.

Preparation of BCA Working Reagent (WR):

BCA reagent was prepared by mixing BCA reagent A and BCA reagent B in the ratio of 50:1 (Reagent A:B).

The following formula was used to determine the total volume of WR required:

(# standards + # unknowns) x(# replicates) x (volume of WR per sample) = total volume of WR required.

Microplate Procedure:

1. 10 microliters of each standard or unknown sample replicate were taken in a microplate well.
2. 200 microliters of the WR were added to each well and the solutions were mixed by placing the plate on a plate shaker for 30 seconds.
3. The plate was then covered and incubated at 37 °C for 30 minutes.

4. The plate was cooled to room temperature and the absorbance was measured at 562 nm on a plate reader.

Determination of Reducing Sugar

Activity of CBH I, CBH II and EG II on cotton fibers was estimated by determining the amount of reducing sugars present in supernatant by the dinitrosalicylic (DNS) acid method [97].

Principle of DNS Method:

The DNS method is used to test the presence of free carbonyl groups (C=O), or the reducing sugars as they are called, produced by the action of cellulolytic enzymes such as cellulases. A free carbonyl group present in a sugar, for example glucose, is oxidized to a carboxyl group and simultaneously 3,5-dintrosalicylic acid (DNS) is reduced to 3-amino, 5-nitrosalicylic acid under alkaline conditions. The color produced by 3-amino, 5-nitrosalicylic acid is measured at 575 nm.

Preparation of DNS solution:

DNS solution used in detecting reducing sugars is prepared as follows:

1. Dinitrosalicylic acid Reagent Solution, 1%
    a. Dinitrosalicylic acid – 10g
    b. Potassium Sodium Tartrate Solution, 300g
    c. Sodium hydroxide – 10g

d. Water to make the volume to 1 liter

Procedure:

1. 100 microliters of standard or unknown solution is measured and added to 100 microliters of deionized water and 400 microliters of DNS reagent in a 5 ml test tube.
2. The test tubes are covered with an aluminum foil to prevent loss of liquid due to evaporation and heated at 90 °C for 5 minutes.
3. After 5 minutes, the test tubes are cooled to room temperature in a cold water bath and the absorbance is measured at 575 nm.
4. The concentration of reducing sugars is determined using a standard curve obtained for a range of cellobiose solutions.

Measurement of Exoglucanase activity

Exoglucanase activity was measured by p-nitrophenyl-β-D-cellobioside method (p-NPC method) [98].

Principle of p-PNC method:

p-nitrophenyl-β-D-cellobioside (pNPC) can be used to measure the activity of exoglucanase. Exoglucanases act on an agluconic bond between p-nitrophenyl and the disaccharide moiety where as endoglucanases and β-glucosidase act on agluconic as well as holosidic bonds. Exoglucanases act on pNPC molecule as shown in the reaction [98]:

p-NP-glu-glu + endoglucanase and β-glucosidase = p-NP + glu

p-NP-glu-glu + exoglucanase = p-NP + glu-glu

where glu is a single molecule of glucose. Interference due to endoglucanase can be compensated for by prior standardization of the assay procedure with a purified endoglucanase.

1. 0.5 milliliters of 1 mM solution of pNPC solution was mixed with a known volume of enzyme solution and incubated for 5 min at 50°C.
2. After 30 minutes 0.5 milliliters of 1% sodium carbonate was mixed with the above solution.
3. The amount of p-nitrophenol produced during the reaction is estimated spectrophotometrically at 410 nm. The color produced due to p-nitrophenol is proportional to the amount of exoglucanase present in the solution.

Application of adsorption isotherms

Different adsorption isotherms were tried to be fitted to the experimental adsorption data. Linear regression analysis was conducted to determine the goodness of fit of the different adsorption isotherms. For Linear adsorption isotherm, a plot of [E] v/s [A] was plotted. In the case of the Langmuir adsorption isotherm, plots of 1/ [E] against 1/ [A] and [E] v/s [E]/[A] were plotted. For Freundlich isotherm, a plot of ln[E] against ln[A] was plotted. The last model that was investigated was the Combined Langmuir-Freundlich isotherm. In this case, a plot of 1/ $[E]^{(1/m)}$ against 1/ [A] was plotted, where $m>1$. In the present case the value of m used was 2.

Linear regression analysis was performed to determine whether the given model is a good fit of the experimental adsorption data. Based on the r-square values, the model that explains the experimental adsorption data the best was selected.

Chapter 4

RESULTS AND DISCUSSION

The results and discussion is discussed in two parts; purification of the enzymes is discussed in the first part and the adsorption of the enzymes on cotton fibers and the fitting of different adsorption models is discussed in the second part.

Part I

Separation of CBH I, II and EG II:

Celluclast was first desalted using a desalting column, HiPrep 26/10. A profile of the enzyme solution desalted on a desalting column is shown in figure 2. In figure 2, the x-axis is the volume of buffer solution collected in fractions during the desalting process and y-axis is the conductance of the solution and the optical density of the solution eluted from the Hiprep 26/10 column measured at 280 nm. The solid line on the plot is the amount of protein eluted from the column whereas the broken line represents the salt eluted from the column. The desalting column efficiently separated the protein from the impurities such as salt present in the solution. The desalting

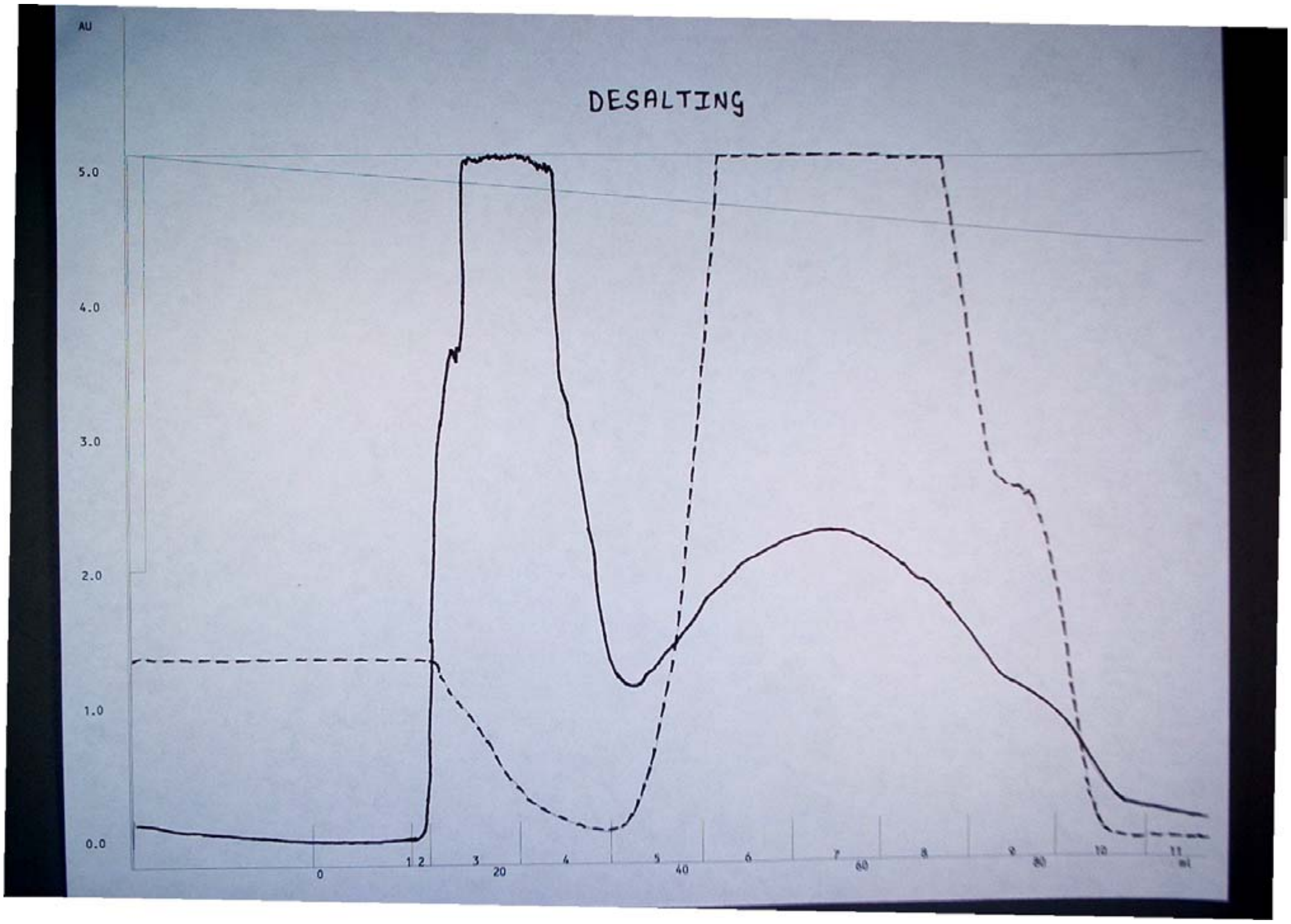

Figure 2. Profile of Enzyme Desalting Process on a Desalting Column

column was used at different stages of purification of the enzyme solution.

The desalted Celluclast was then injected onto a DEAE Sepharose CL-6B column. The enzyme fraction unadsorbed on the DEAE Sepharose column was eluted along with the starting buffer A. This enzyme fraction unadsorbed on the DEAE Sepharose column may contain CBH II and EG II. CBH I which was adsorbed on the DEAE Sepharose CL-6B column was eluted from the column by the salt gradient developed by buffer B.

Figure 3 is the elution profile of CBH I under the salt gradient created by buffer B. The fractions showing significant amounts of protein were collected, tested for exoglucanase activity using the p-nitrophenyl-β-D-cellobioside method and mixed together [98]. The eluted fraction was then precipitated, desalted and concentrated before storing at 3 °C.

The fraction unadsorbed on DEAE Sepharose CL-6B was collected, precipitated, desalted and then injected onto the SP Sepharose XL column. CBH II fraction was adsorbed on the SP Sepharose XL column while the EG II fraction is eluted along with buffer A. The adsorbed CBH II fraction was then eluted under a salt gradient, collected, concentrated by precipitation and dialysis and stored at 3 °C until used for adsorption experiments.

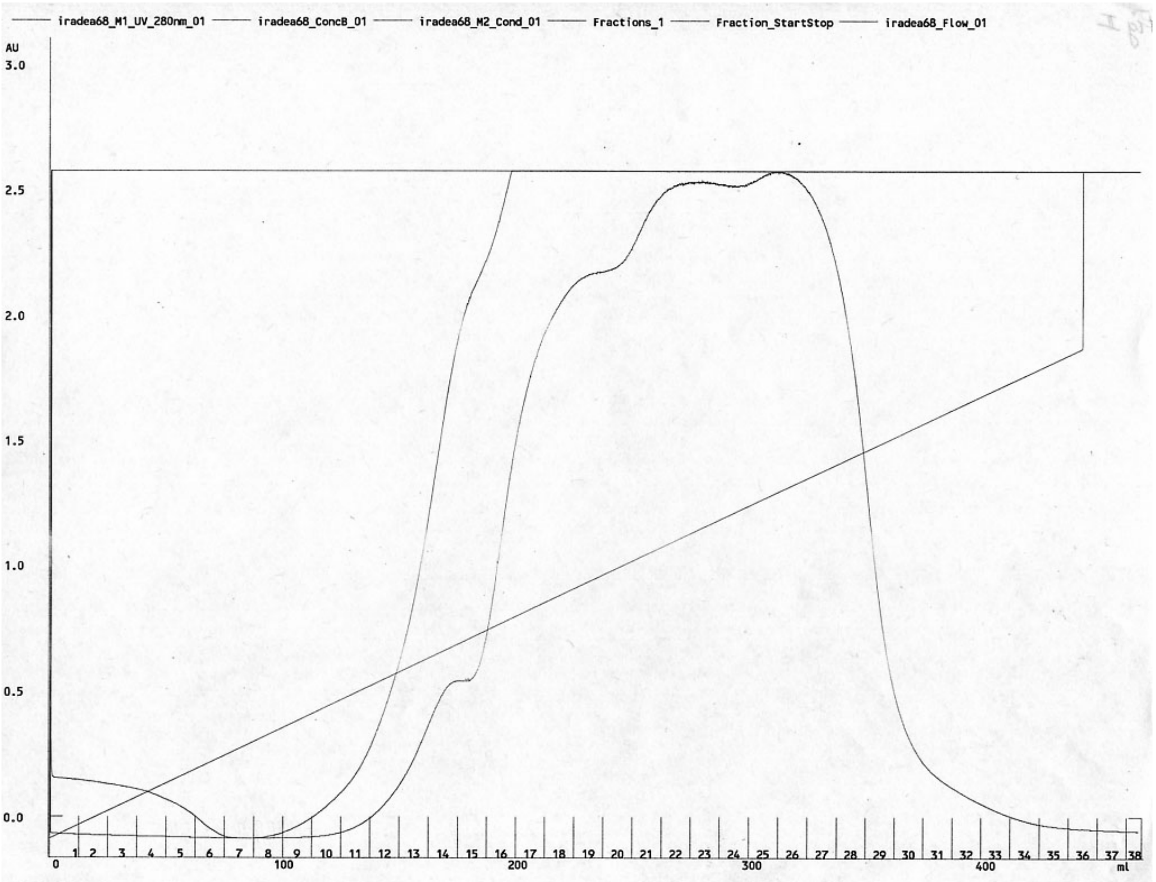

Figure 3. Profile of elution of adsorbed fraction (CBH I) on DEAE Sepharose CL-6B column.

EG II fraction unadsorbed on SP- Sepharose XL may have contained some impurities and hence was injected to Mono S HR 10/10 column. EG II fraction was then eluted at low salt concentration from the column. The fraction was collected, concentrated by precipitation and dialysis and stored at 3 °C until used for adsorption experiments.

Determination of the Purity of the enzymes:

The purity of the isolated enzymes was checked by SDS PAGE. The SDS PAGE was prepared as described in the materials and methods chapter. Figure 4 is a photograph of a SDS PAGE prepared for the three enzymes, CBH I, CBH II and EG II along with a low molecular weight marker protein that is a standard used in the SDS PAGE. A single solid band indicates that the protein is pure enough. From figure 4, indicates single bands for all the three enzymes, and hence that they are pure enzymes.

Part II

The adsorption data was evaluated by linear regression to determine the adsorption model that best explains the adsorption data. Four different adsorption models tested were: Linear, Langmuir, Freundlich and Combined Langmuir-Freundlich models. The following were plotted to test the fit of the four models to the experimental adsorption data obtained.

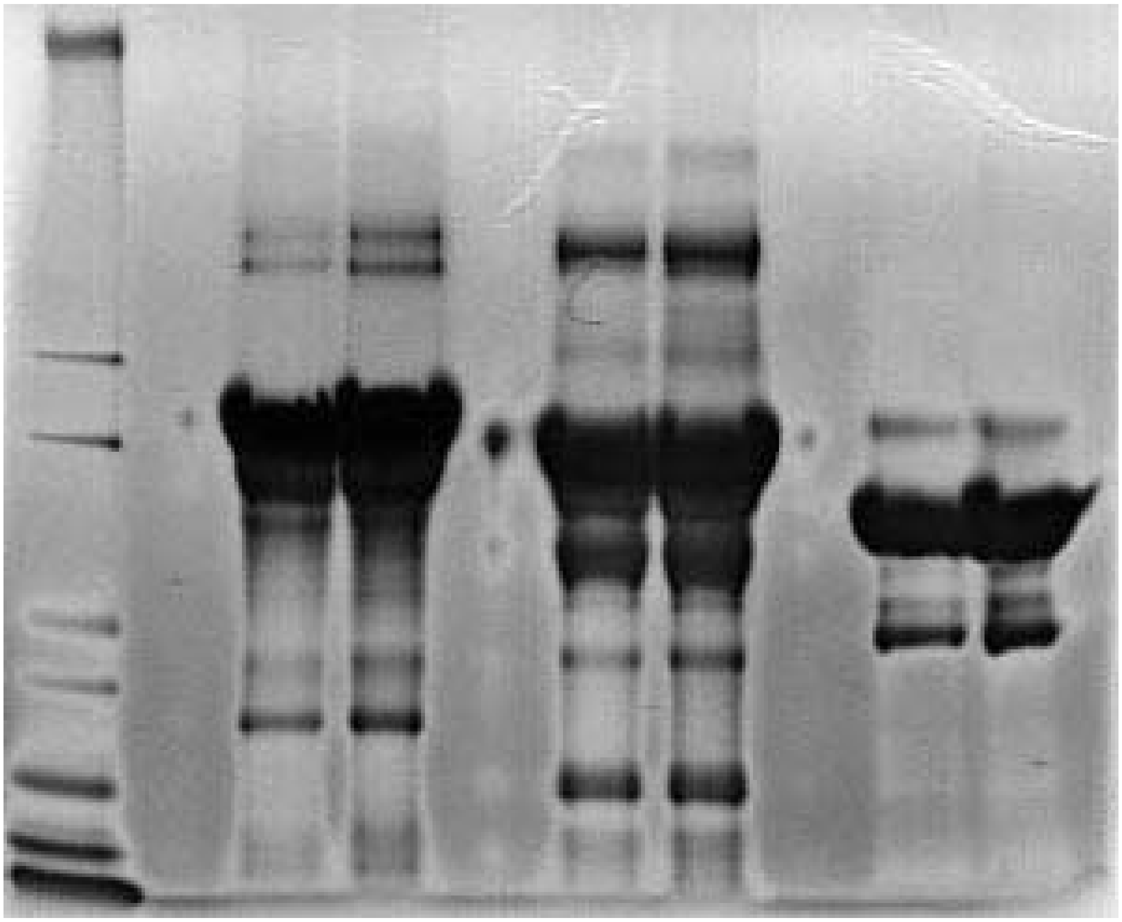

Figure 4. SDS PAGE to determine the purity of CBH I, CBH II and EG II enzymes.

1. [E] v/s [A] for Linear model
2. [E] v/s [E]/[A] for Langmuir model suggested by Kim et.al. [81]
3. 1/[E] v/s 1/[A] for Langmuir model
4. ln[E] v/s ln[A] for Freundlich model
5. $1/[E]^{1/m}$ v/s 1/[A] for Combined Langmuir-Freundlich model

In the plots mentioned, [E] is the amount of enzyme present in the solution before adsorption and [A] is the amount of enzyme adsorbed on cotton fibers per gram of cotton fibers. The first plot was used to test whether the linear model was a good fit for the adsorption data of the CBH I, CBH II and EG II. Peitersen et.al [74] and Kim et.al.[81, 82] determined whether adsorption of cellulases on microcrystalline cellulose can be explained by Langmuir model by plotting the second plot. Hence in the present investigation the second plot was used to determine whether the model described by Peitersen et.al.[74] and Kim et.al.[82] can explain the adsorption data. The third plot in the above list determines whether the adsorption data of the enzymes is explained by Langmuir adsorption isotherm [99]. The fourth plot tested the goodness of fit of the Freundlich model and the last plot was plotted to test the combined Langmuir-Freundlich Model for the adsorption of CBH I, CBH II and EG II on cotton fibers.

The R-square values of the linear regression performed were used to determine whether the model was a good fit for the given experimental adsorption data. A R-square value close to 1 indicates a good fit by the model for the given experimental data whereas a R-square value near 0 indicates that the model is not a good fit for the given experimental data.

The results of the adsorption study are discussed separately for CBH I, CBH II and EG II. The adsorption data points for CBH I, CBH II and EG II are the mean values calculated from triplicate experimental measurements.

CBH I:

Figure 5 is a plot of the amount of enzyme adsorbed/ gram of cotton (A) against the enzyme present in the treatment bath (E in mg/ml). An increase in adsorption of CBH I was observed with an increase in concentration of CBH I present in the solution indicating that there are enough sites of adsorption on cotton fibers for CBH I. Increase in adsorption of CBH I with increase in concentration of CBH I in the solution was observed at all treatment temperatures studied, 25, 35, 45 and 55 °C. On plotting the amount of enzyme adsorbed on cotton fiber at different temperatures, as shown in figure 6, it can be seen that temperature of the adsorption experiments influences the adsorption of CBH I onto cotton fibers to a small extent. The

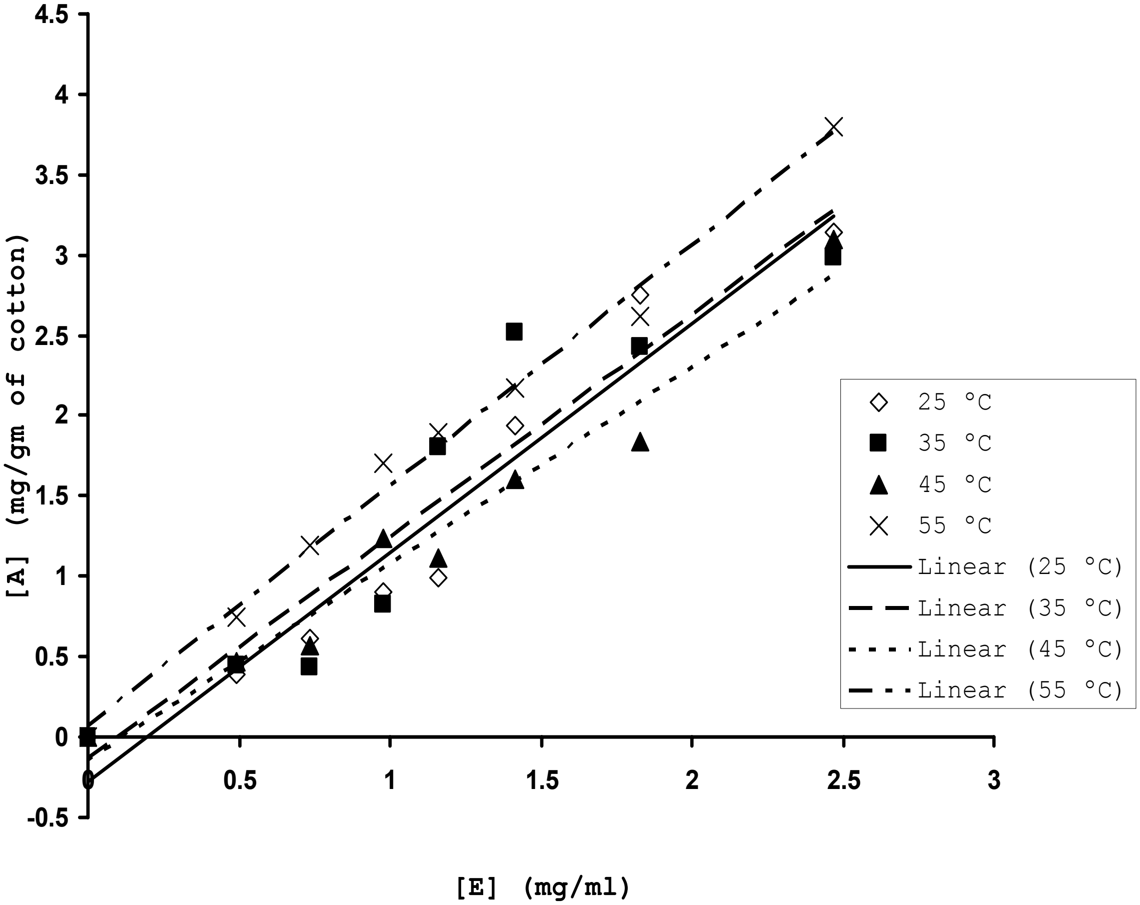

Figure 5. Linear plot for the adsorption isotherms of CBH I on cotton fibers.

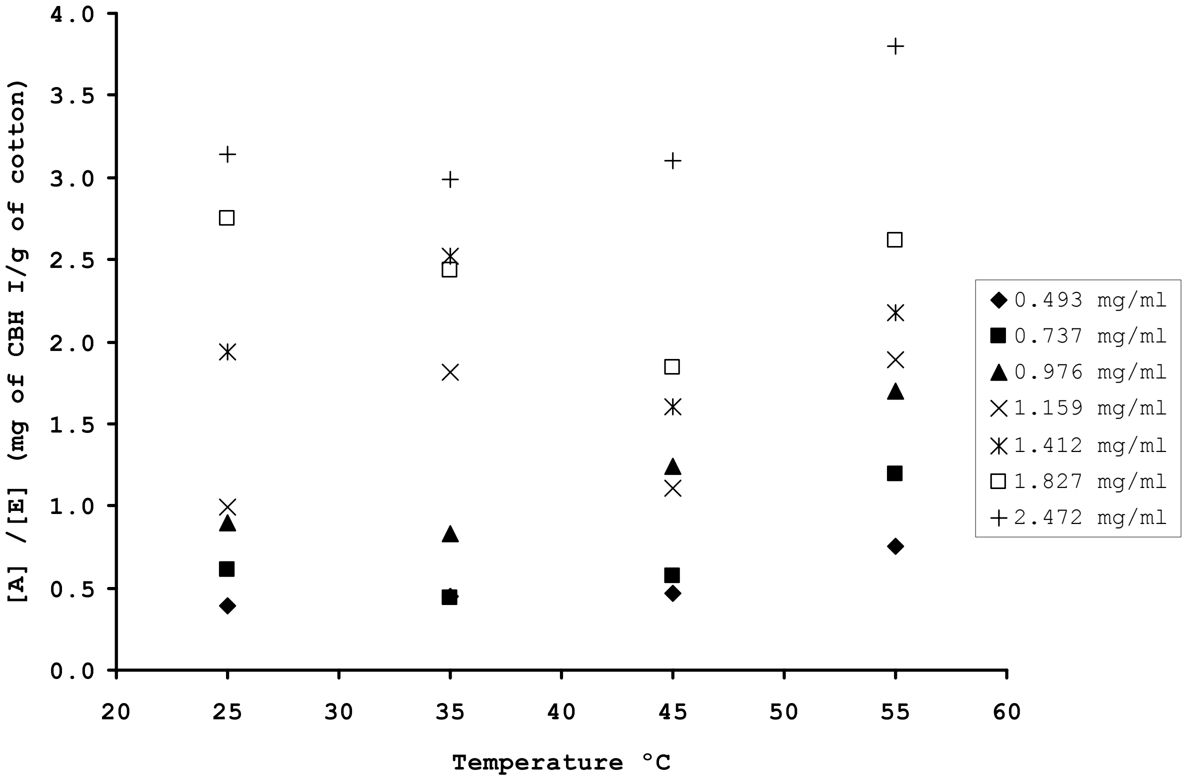

Figure 6. Effect of temperature on adsorption of CBH I on cotton fibers at a given concentration of CBH I

adsorption of CBH I on cotton fibers at 55 °C was higher as compared to the other temperatures studied at all given concentrations of CBH I in the treatment baths.

The ratio of the amount of CBH I adsorbed on cotton fibers [A] and CBH I remaining in the enzyme bath [Es] at different enzyme concentrations was plotted against temperature of adsorption in figure 7. The amount of CBH I adsorbed on cotton fibers at equilibrium is higher at 55 °C than the other adsorption temperatures studied irrespective of the concentration of CBH I in the treatment bath.

Linear trend lines for adsorption of CBH I at each temperature have been plotted in figure 5. Linear regression analysis was performed on the linear adsorption model given by equation 1. The output of the statistical analysis performed by SAS is included as Appendix A. The R-square values for the linear model are listed in Table 4. From the R-square values for the adsorption data at different temperatures, it can be seen that the R-square values are greater than 0.9 except for the adsorption of CBH I on cotton fibers at treatment temperature of 35 °C indicating that the linear adsorption model is a good fit for the experimental adsorption data.

Plot of [E] v/s [E]/ [A]

Studies involving adsorption of cellulases on microcrystalline cellulose used a plot of [E] against [E]/[A] to

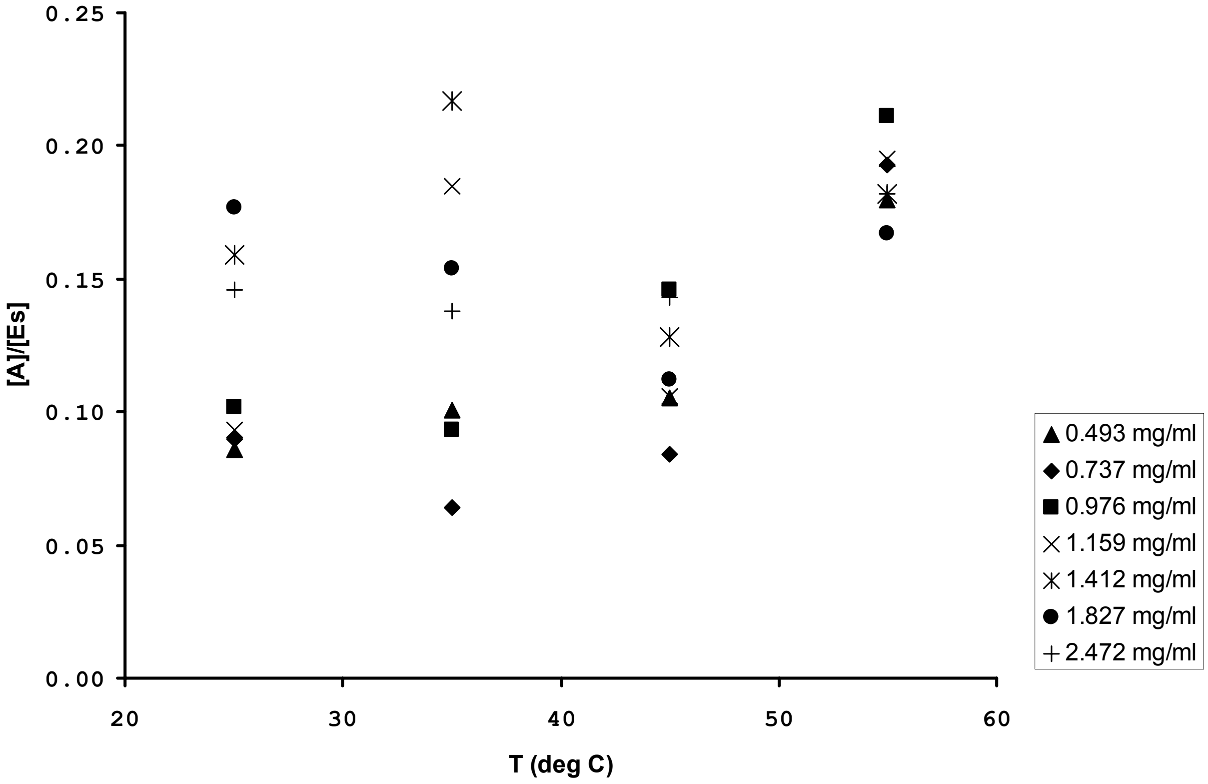

Figure 7. Effect of temperature on equilibrium adsorption of CBH I on cotton fibers at a given concentration of CBH I

Table 4. R-square values for the different plots for the experimental adsorption data of CBH I

Plot	**25 °C**	**35 °C**	**45 °C**	**55 °C**	**Average**
[E] v/s [A] (Linear)	0.9422	0.8829	0.9648	0.9917	0.9851
1/[E] v/s 1/[A] (Langmuir)	0.9868	0.8278	0.9349	0.9898	0.9802
[E] v/s [E]/[A] (Langmuir by Kim et.al.[81])	0.6717	0.2899	0.3054	0.1730	0.4928
Ln[E] v/s ln[A] (Freundlich)	0.9639	0.8614	0.9407	0.9877	0.9814
$1/[E]^{1/m}$ v/s 1/[A] Combined Langmuir-Freundlich)	0.9703	0.8361	0.9277	0.9491	0.9628

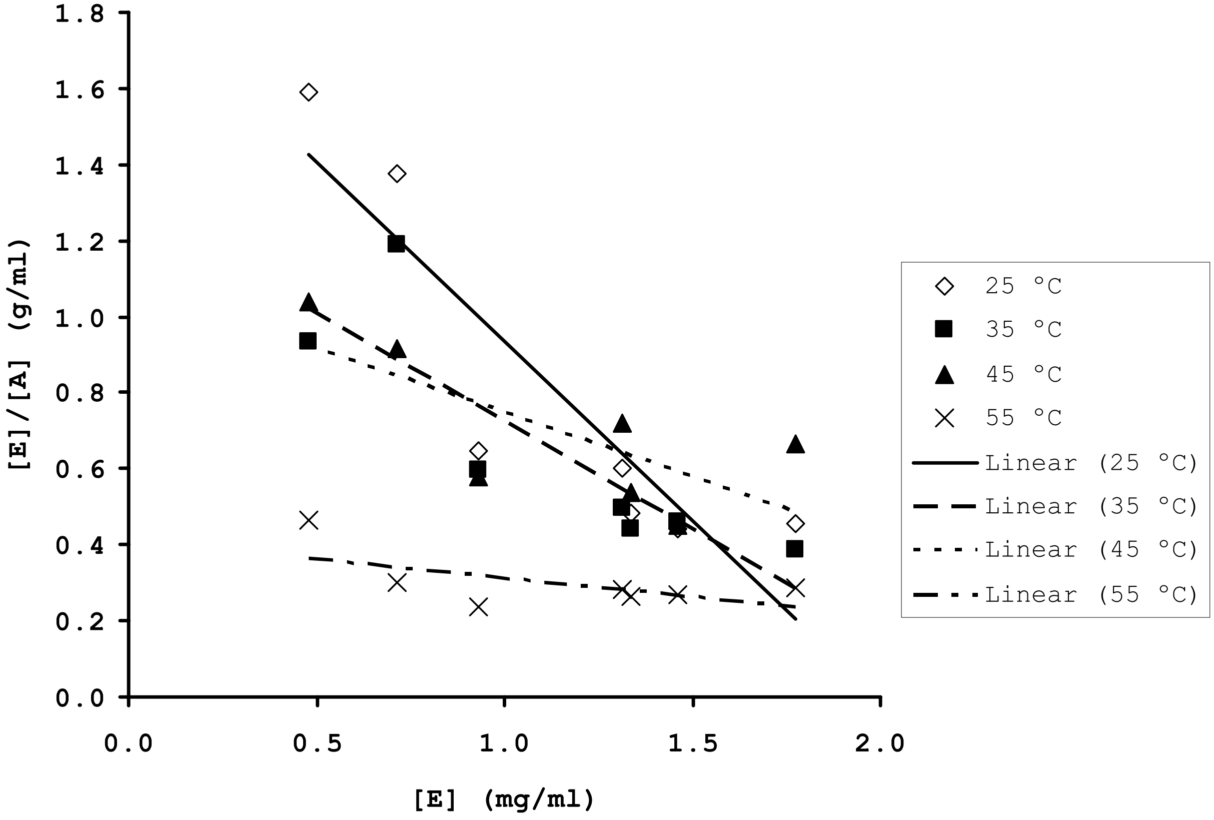

Figure 8. Langmuir plot for the adsorption isotherms of CBH I on cotton fibers (Method I)

determine if the adsorption data was explained by a Langmuir adsorption model [81]. In the present study, [E] was plotted against [E]/[A] was plotted as shown in figure 8. Adsorption data for CBH I on cotton fibers at 25, 35, 45 and 55 °C were plotted. The trend lines for the adsorption data of CBH I at different temperatures are also plotted in the figure 8.

Plot of 1/ [E] v/s 1/ [A]

Another method to determine whether the adsorption data obtained can be explained by a Langmuir adsorption isotherm is to plot values of 1/[E] against 1/[A] [99]. A plot of 1/[E] against 1/[A] is given in figure 9 where [E] is the amount of CBH I present in the bath before adsorption and [A] is the amount of CBH I adsorbed on cotton fibers under the given conditions. Trend lines for the adsorption data at different temperatures are plotted in figure 9.

Plot of ln[E] v/s ln[A]

Figure 10 shows the plots of natural logarithms of [E] against [A] for the adsorption of CBH I at different temperatures on cotton fibers. The trend lines for adsorption at the different temperatures studied are shown.

Plot of 1/ $[E]^{1/m}$ v/s 1/[A]

A plot of 1/ $[E]^{1/m}$ v/s 1/[A] was plotted in order to determine whether the adsorption data can be explained by a combined Langmuir-Freundlich isotherm. The combined

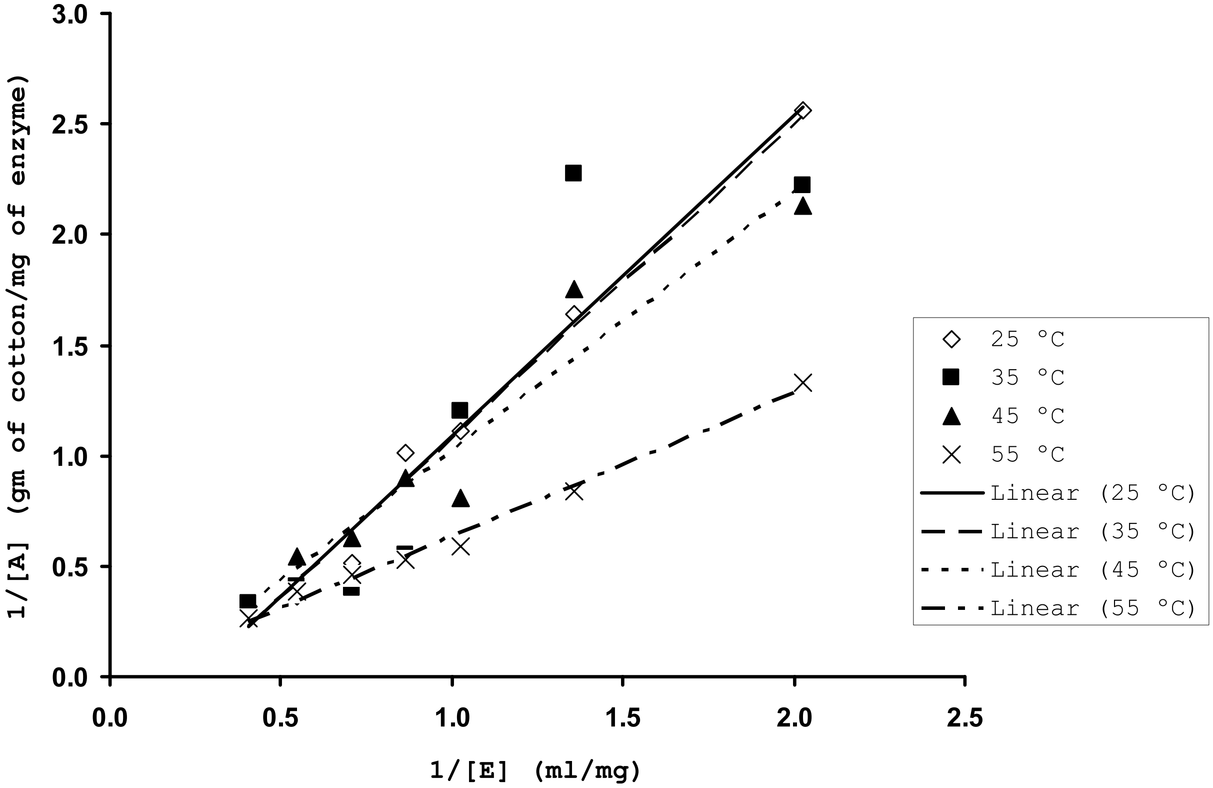

Figure 9. Langmuir plot for the adsorption isotherms of CBH I on cotton fibers (Method II)

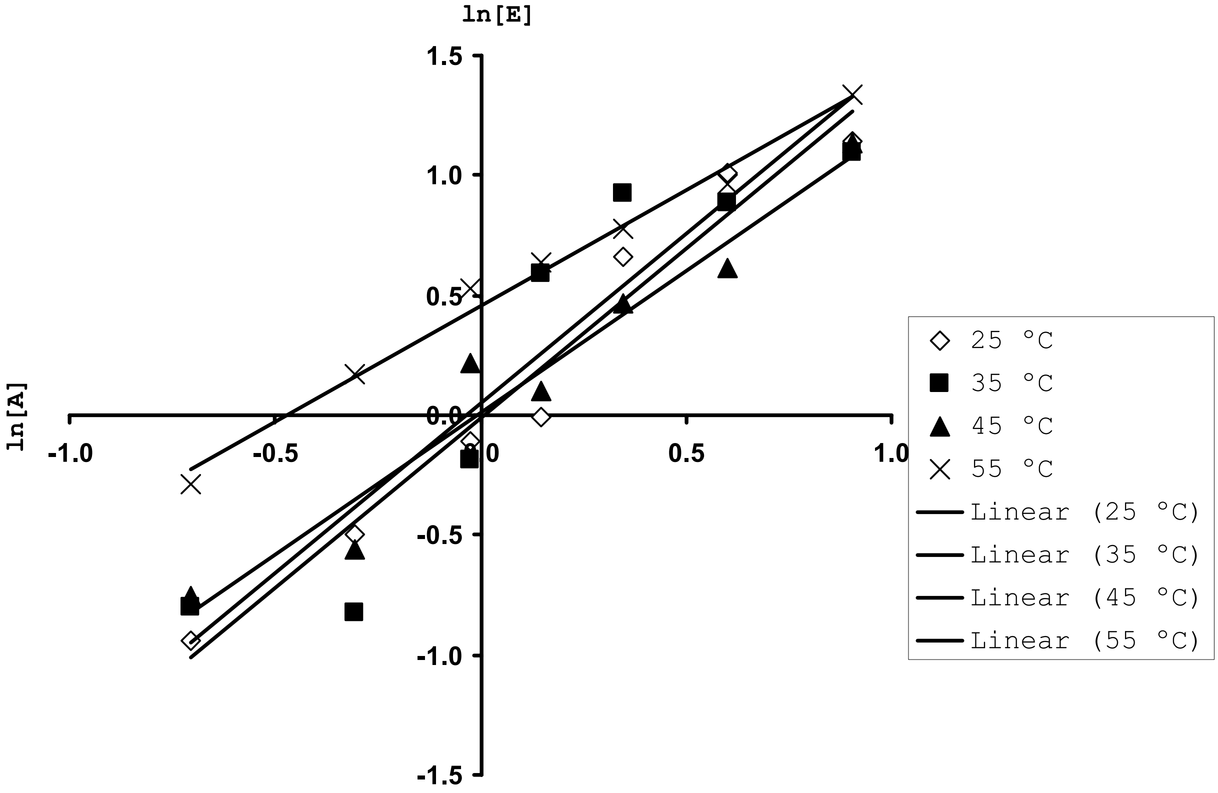

Figure 10. Freundlich plot for the adsorption isotherms of CBH I on cotton fibers

Langmuir-Freundlich isotherm tries to account for the inadequacies of the Langmuir and Freundlich models. The model is empirical and hence the parameters obtained from the model do not have any physical significance. Figure 11 is the plot of the values of $1/ [E]^{1/m}$ against 1/[A] where m=2.

Linear regression was conducted on the adsorption data of CBH I to determine which of the adsorption models fit the experimental adsorption data. The R-square values for the different regressions models are listed in Table 4. The R-square values for the different models were used to determine if the model is a good fit of the present adsorption data. The regression analysis output for adsorption of CBH I at different temperatures is given as Appendix A.

For the linear adsorption model, the R-square values listed in Table 4 were found to be greater than 0.9 except for the plot at 35 deg C indicating that the linear adsorption model is a good fit for the experimental adsorption data. Hence the linear adsorption model could be used to describe the adsorption behavior of CBH I on cotton fibers within the given concentration range and experimental conditions.

The R-square values for Langmuir adsorption model based on the plots of [E] against [E]/[A] listed in Table 4 are found to be close to zero on a scale of 0 to 1 implying that the Langmuir adsorption model as described by Kim et.al.[81] is not a good

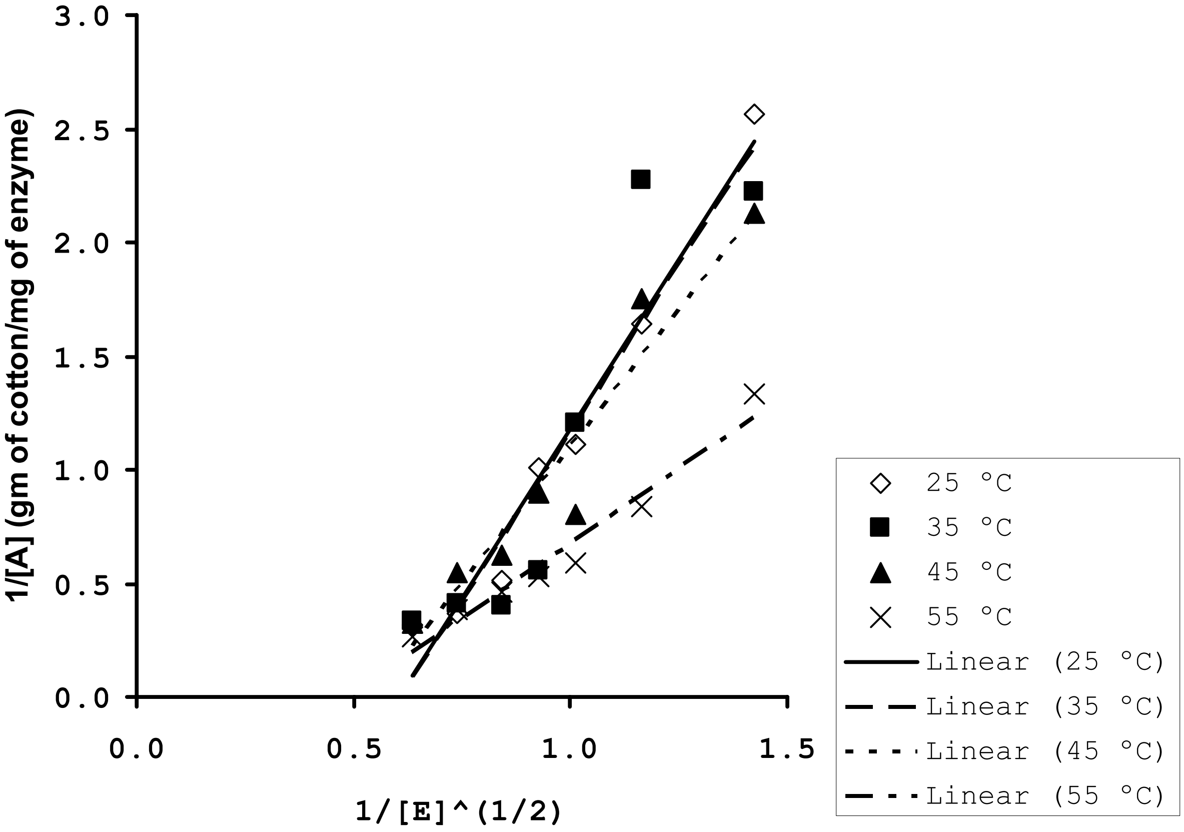

Figure 11. Combined Langmuir-Freundlich plot for the adsorption isotherms of CBH I on cotton fibers

fit for the experimental adsorption data of CBH I. Hence the present adsorption data cannot not be explained by Langmuir adsorption model described by Kim et. al. [81] based on the plots of [E] against [E]/[A]. When linear regression was conducted using plots of 1/[E] against 1/[A], it was found that the R-square values were closer to 1 indicating that the Langmuir adsorption model is a good fit for the adsorption data of CBH I. Hence, the present experimental adsorption data for CBH I can be explained by Langmuir adsorption model based on the plots of 1/ [E] against 1/[A].

The Freundlich adsorption model can be said to be a good fit for the given experimental adsorption data since the linear regression of ln[E] against ln[A] gave R-square values in the range of 0.8614 to 0.9877 for the four temperatures at which adsorption experiments were conducted.

The R-square values for the combined Langmuir-Freundlich model with m=2 are listed in Table 4. The values for the adsorption data at 25, 45 and 55 °C are greater than 0.9 whereas the R-square value for the adsorption data for 35 °C is 0.8361 indicating that the combined Langmuir-Freundlich model could be used to describe the adsorption data of CBH I.

In the adsorption studies conducted by Kim et al and Peiterson et al [74] [82], the authors tried to determine whether the experimental adsorption data could be explained by

Langmuir adsorption data alone. In the present study, the experimental adsorption data was tested for more than one type of adsorption models. Kim et al,[82] reported that the adsorption of CBH I on microcrystalline cellulose was explained by Langmuir adsorption isotherm. However, in the present data, though the range of enzyme concentration used in the present study is similar, the adsorption data could not be explained by the Langmuir adsorption model as employed by Kim et.al.[82] and Peitersen et.al [74], i.e. plot a graph of [E] against [E]/[A]. But when a plot of the reciprocals of [E] and [A] were plotted, the adsorption data could be explained by Langmuir adsorption model. The experimental data is explained by linear, Freundlich and combined Langmuir-Freundlich models.

The average of the values of adsorption of CBH I at each given concentration on cotton fibers at different temperatures was plotted and linear regression analysis was conducted to determine the best fitting model for the data. In Table 4, it can seen that the R-square values for all the models are greater than 0.9 except for the Langmuir model when tested by plotting [E] against [E]/[A] (model described by Kim et.al.[81]). R-square values close to 1 for more than one model indicate that the experimental adsorption data could be explained by different models at the given range of concentration.

CBH II:

Plot of [E] v/s [A]

Figure 12 is a plot of the amount of CBH II adsorbed per gram of cotton against the amount of CBH II present in the original solution. The amount of CBH II adsorbed on the cotton fibers increases with the concentration of CBH II initially present in the treatment bath. The adsorption of CBH II at the different temperatures studied, 25, 35, 45 and 55 °C was higher at 55 °C as compared to the adsorption at the other adsorption temperatures studied. Figure 13 shows the effect of temperature on adsorption of CBH II at a given concentration of CBH II. Linear trend lines are shown in each figure for adsorption of CBH II from solutions of different concentrations at each treatment temperature.

Figure 14 is a plot of the ratio of amount of CBH II adsorbed on cotton fibers [A] and the amount of CBH II remaining in the enzyme bath [Es] after adsorption at different enzyme concentrations against temperature of adsorption. From figure 14 it is evident that the amount of CBH II adsorbed on cotton fibers at equilibrium is higher at 55 °C compared to other treatment temperatures studied irrespective of the concentration of CBH II.

Plot of [E] v/s [E]/ [A]

Figure 15 is a plot of the amount of CBH II present in

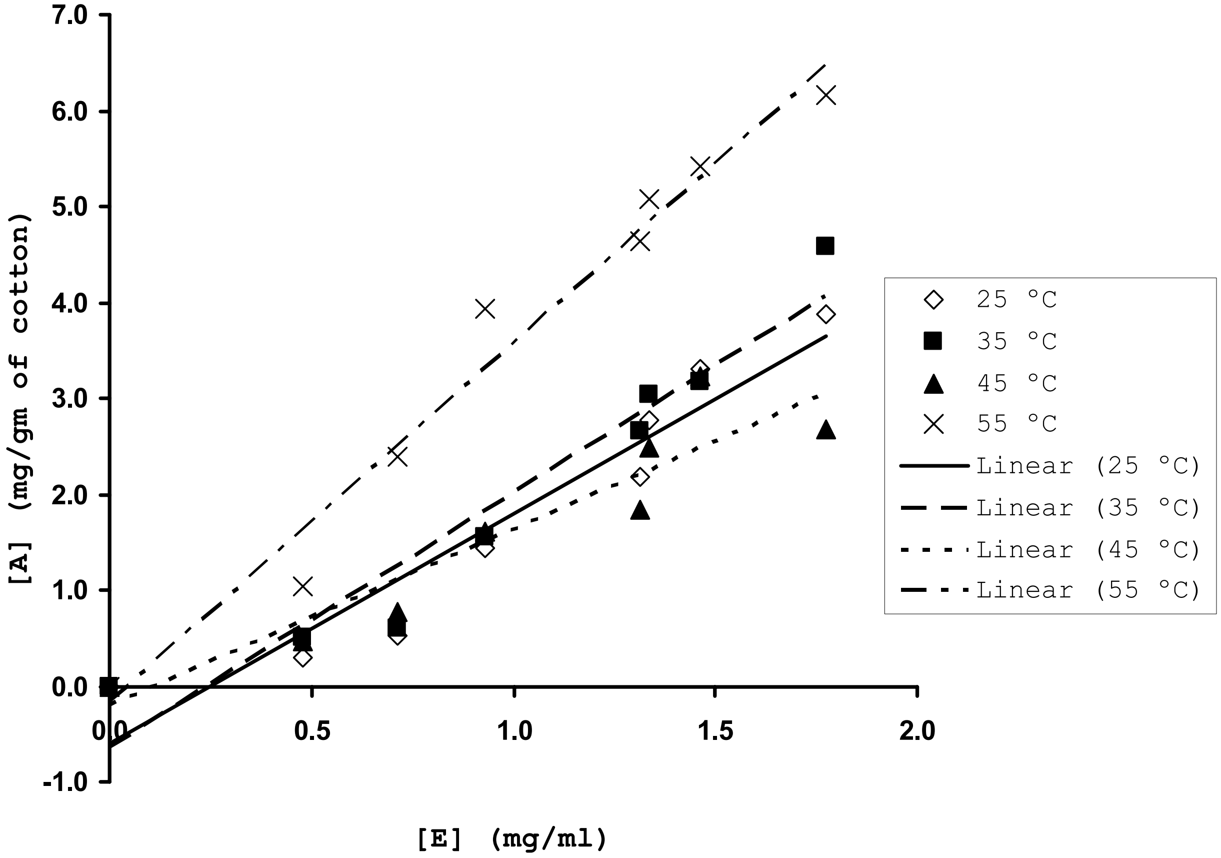

Figure 12. Linear plot for the adsorption isotherms of CBH II on cotton fibers.

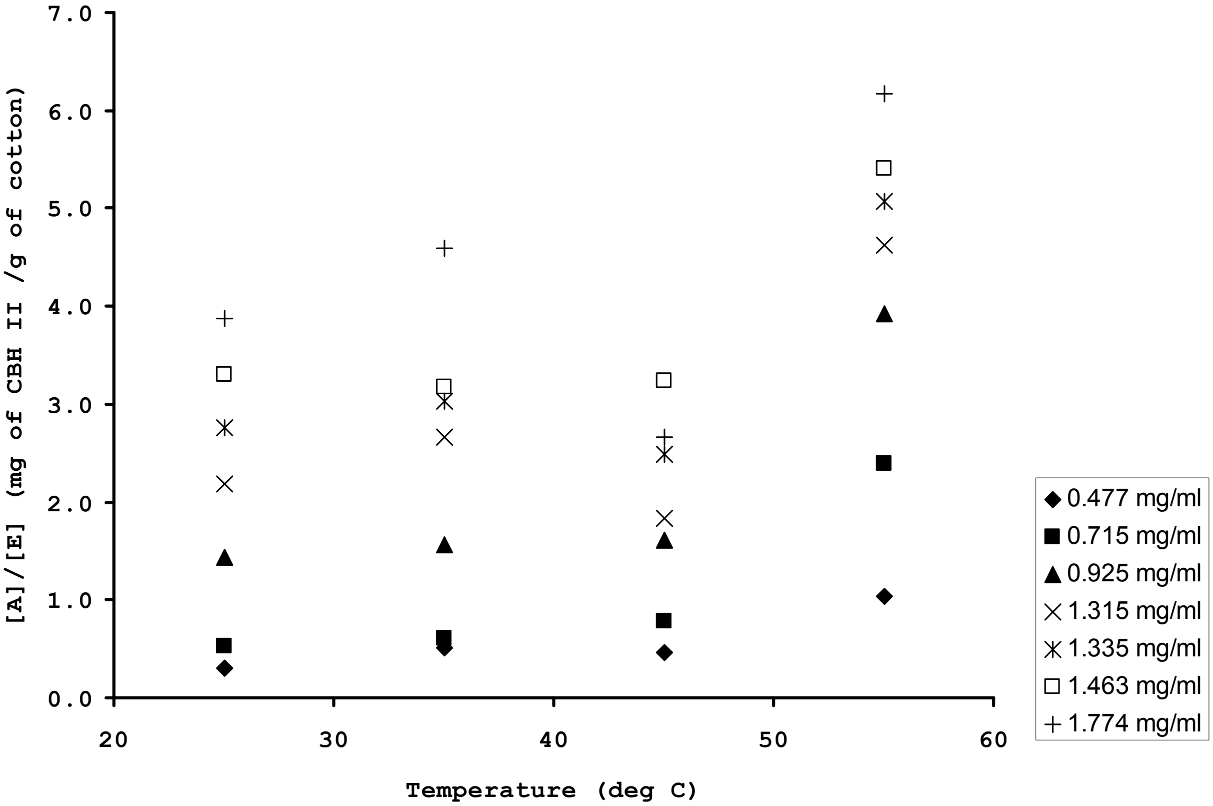

Figure 13. Effect of temperature on adsorption of CBH II on cotton fibers at a given concentration of CBH II

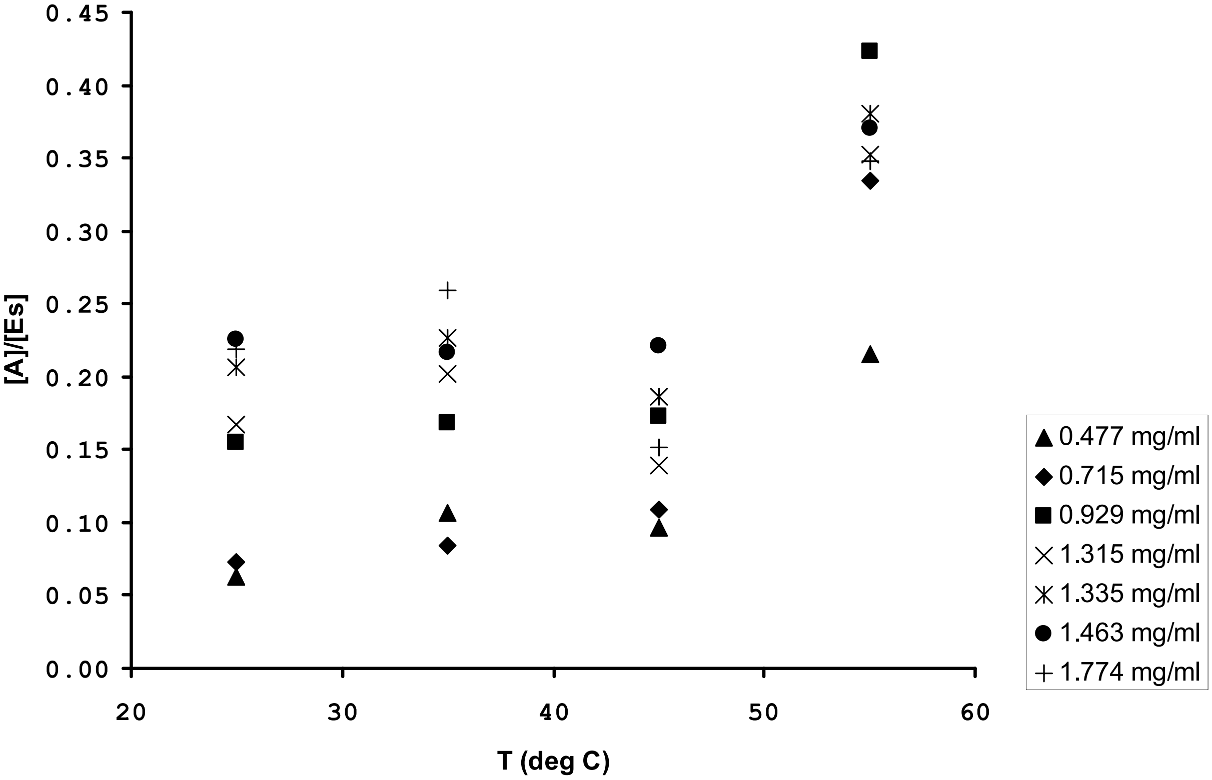

Figure 14. Effect of temperature on equilibrium adsorption of CBH II on cotton fibers at a given concentration of CBH II

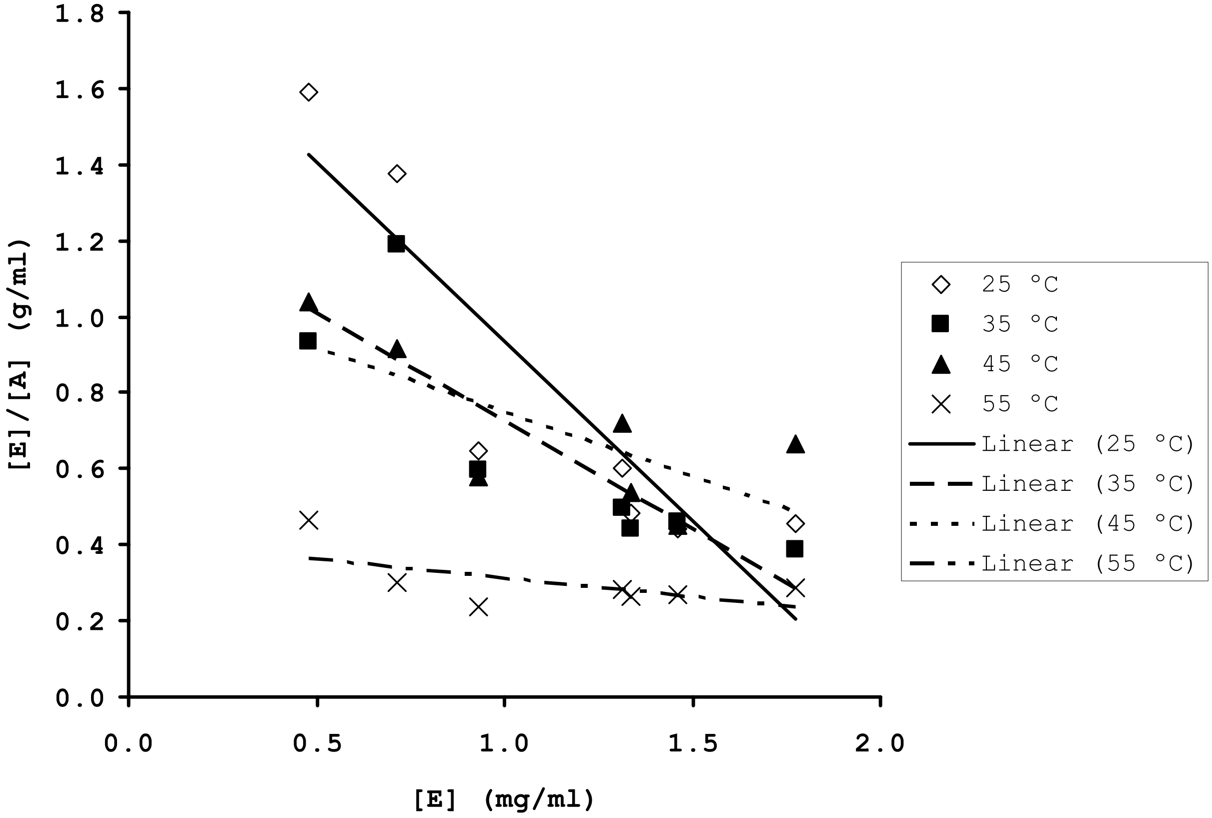

Figure 15. Langmuir plot for the adsorption isotherms of CBH II on cotton fibers (Method I)

solution against the ratio of the amount of CBH II present in solution to the amount of CBH II adsorbed per gram of cotton fibers. Plotting [E] against [E]/[A] is the method used by Kim et.al. [81] to determine whether the experimental adsorption data is explained by the Langmuir adsorption model.

Plot of 1/[E] v/s 1/[A]

Figure 16 is a plot of the reciprocal of the amount of CBH II present in solution against the amount of CBH II adsorbed per gram of cotton fibers. By plotting the reciprocals of the [E] and [A], we can determine whether the experimental adsorption data of CBH II can be explained by the Langmuir adsorption model.

Plot of ln[E] v/s ln[A]

Figure 17 is the log-log plot of amount of CBH II present in solution against the amount of CBH II adsorbed per gram of cotton fibers. The plots of natural logarithm values of [E] and [A] are plotted to determine if the experimental adsorption data can be explained by the Freundlich adsorption isotherm.

Plot of $1/[E]^{1/m}$ v/s 1/[A]

Figure 18 is the plot of inverse of the amount of CBH II present in solution to the power of 1/m (where m=2) against the inverse of the amount of CBH II adsorbed per gram of cotton fibers. The linear regression output of the different models for the experimental adsorption data of CBH II is given as

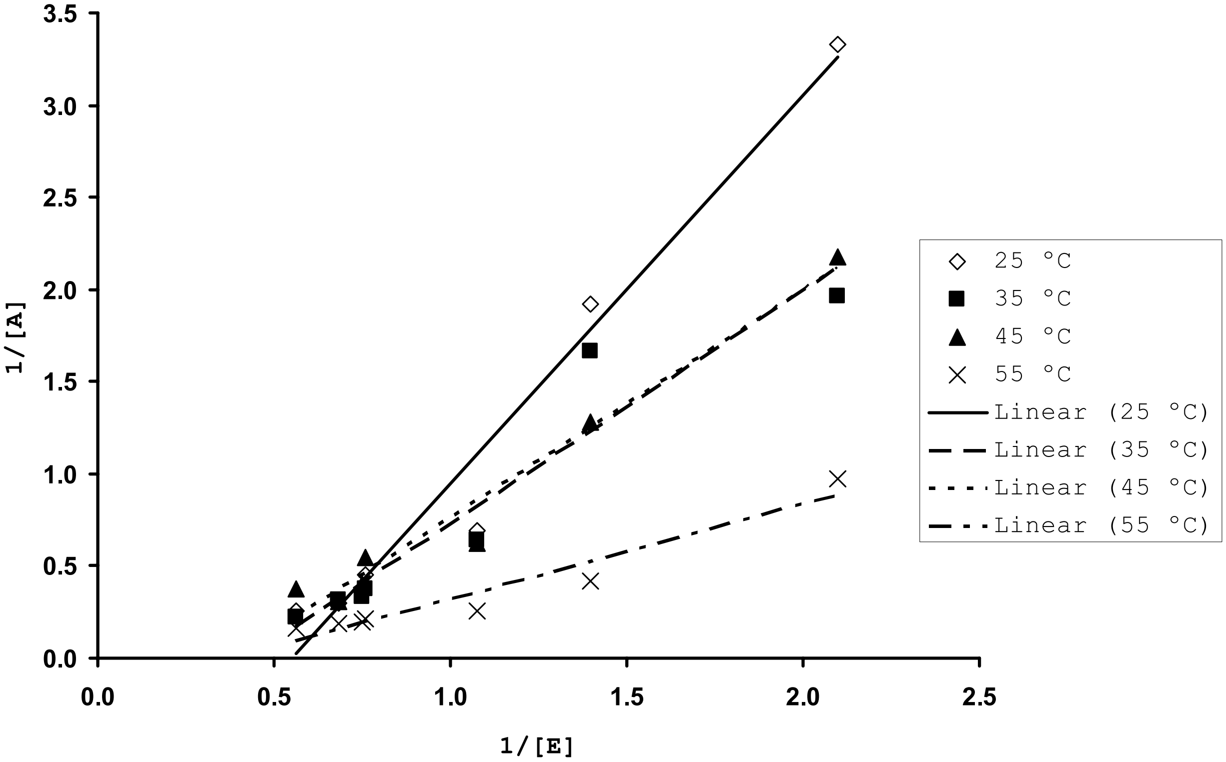

Figure 16. Langmuir plot for the adsorption isotherms of CBH II on cotton fibers (Method II)

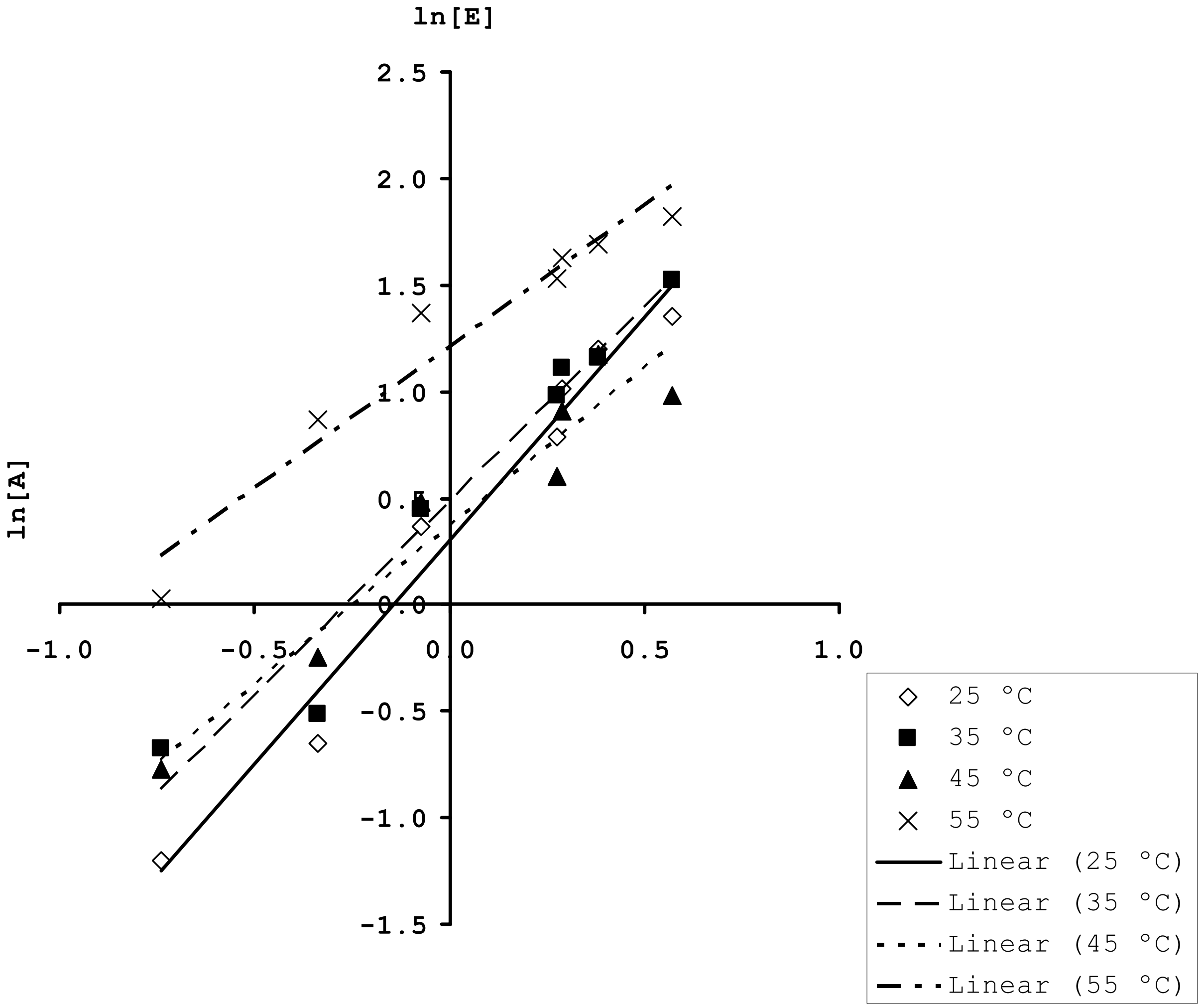

Figure 17. Freundlich plot for the adsorption isotherms of CBH II on cotton fibers

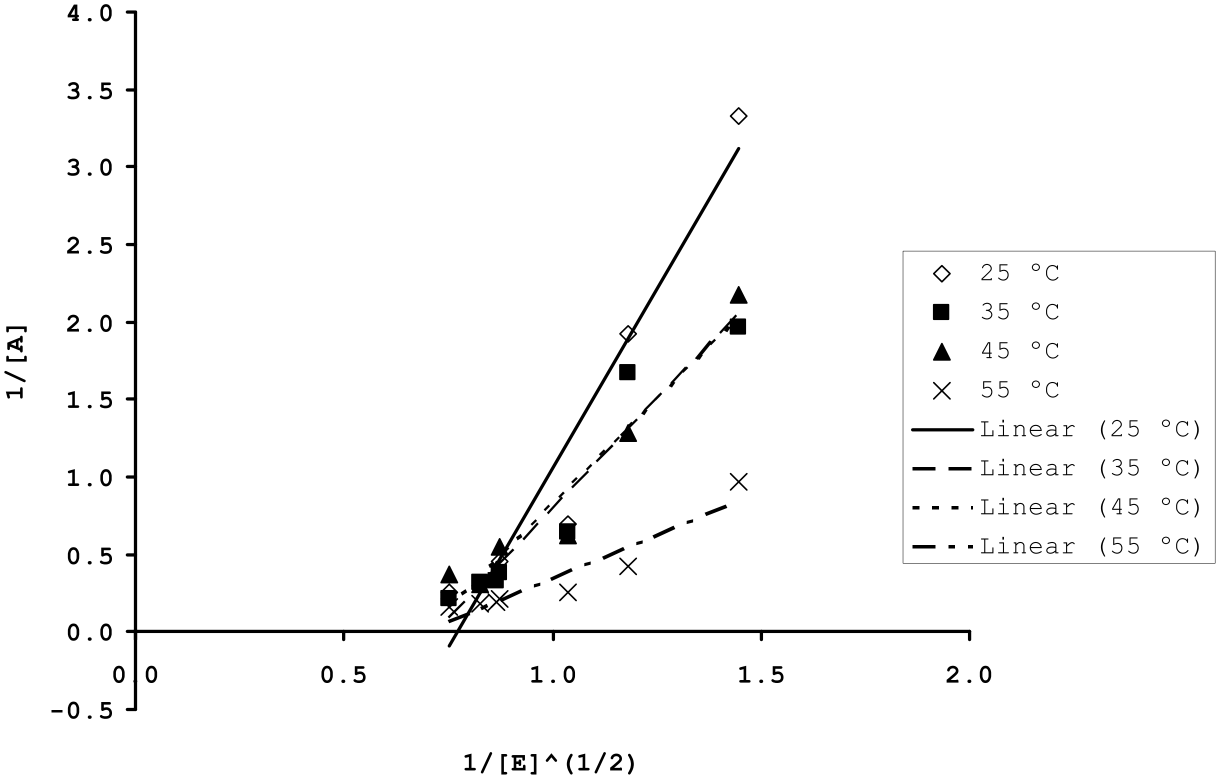

Figure 18. Combined Langmuir-Freundlich plot for the adsorption isotherms of CBH II on cotton fibers

Appendix B.

The R-square values for the regression analysis for the model used to fit the adsorption data for CBH II on cotton fibers are given in Table 5. The R-square values for the linear adsorption model are found to be greater than 0.9 except for adsorption at 45 °C, implying that the linear adsorption model is a good fit for the experimental adsorption data obtained at different temperatures of treatment of cotton fibers with CBH II.

The R-square values of the regression analysis for plots of [E] against [E]/[A] were in the range of 0.5387 and 0.8025 except for the plot of adsorption points at 55°C which was 0.3596 (Table 5). From the R-square values the Langmuir adsorption isotherm as described by Peitersen et.al. [74] and Kim et.al. [81], does not explain the experimental adsorption data. In contrast, plotting the reciprocal values of [E] and [A] resulted in R-square values greater than 0.9 at all treatment temperatures and indicated that the experimental adsorption data can be described by Langmuir adsorption isotherm.

The R-square values for the Freundlich plot, which is a natural logarithmic plot of [E] and [A], are the highest among the different models. The R-square values for the adsorption of CBH II at all the temperatures studied were found to be greater

Table 5. R-square values for the different plots of the experimental adsorption data of CBH II

Plot	**25 °C**	**35 °C**	**45 °C**	**55 °C**	**Average**
[E] v/s [A] (Linear)	0.9213	0.9303	0.8786	0.9723	0.9621
1/[E] v/s 1/[A] (Langmuir)	0.9689	0.9170	0.9660	0.9274	0.9729
[E] v/s [E]/[A] (Langmuir by Kim et.al.[81])	0.8025	0.7259	0.5387	0.3596	0.7228
Ln[E] v/s ln[A] (Freundlich)	0.9714	0.9543	0.9280	0.9402	0.9722
$1/[E]^{1/m}$ v/s 1/[A] Combined Langmuir-Freundlich)	0.9431	0.9255	0.9379	0.8793	0.9406

than 0.9. Hence the Freundlich adsorption model appears to be a good fit of the experimental adsorption data. Similarly, the R-square values for the adsorption plots of CBH II at the different temperatures are greater than 0.9 except the adsorption data at 55 °C, which was 0.8793 and is still acceptable. Hence the Combined Langmuir-Freundlich adsorption model with m=2 is a good fit for the experimental adsorption data.

The experimental adsorption values of CBH II at different concentrations were plotted to determine the effect of increasing temperature on adsorption of CBH II as shown in figure 12. From the figure, it could be said that temperature did not affect the adsorption of CBH II on cotton fibers at a given concentration except at 55 °C at which higher adsorption of CBH II on cotton fibers was observed at all given concentrations. The average values of adsorption values of CBH II on cotton fibers at different temperatures were then plotted. From the R-square values for the regression analysis using the average values (shown in table 5) the R-square values were greater than 0.9 for all plots except for the plot of [E] against [E]/[A] which is Peitersen et.al. [74], and Kim et.al's [82] method to determine whether the Langmuir adsorption model could be used to explain the experimental adsorption data. Hence, from the R-square values, that the experimental adsorption data for CBH II on cotton fibers could be explained by more than one adsorption model.

EG II:

EG II was the third enzyme that was evaluated in this study. The adsorption studies were performed and the experimental adsorption data obtained was then subjected to linear regression. The output of the linear regression is included in the dissertation as Appendix C.

Plot of [E] v/s [A]

The amount of EG II adsorbed per gram of cotton plotted against the amount of EG II initially present in the solution indicates an increasing adsorption of EG II on cotton fibers with increasing concentration of EG II in solution (Figure 19). A graph of EG II adsorbed per gram of cotton for individual concentrations with increasing temperatures is shown in figure 20. This plot shows that the adsorption of EG II is not affected by temperature at low concentrations. At higher concentrations of EG II (1.557, 1.857 and 2.069 mg/ml), an increase in the adsorption of EG II on cotton fibers was observed with increase in temperature of treatment.

Figure 21 is a plot of the ratio of amount of CBH II adsorbed on cotton fibers [A] and the amount of EG II remaining in the enzyme bath [Es] after adsorption at different enzyme

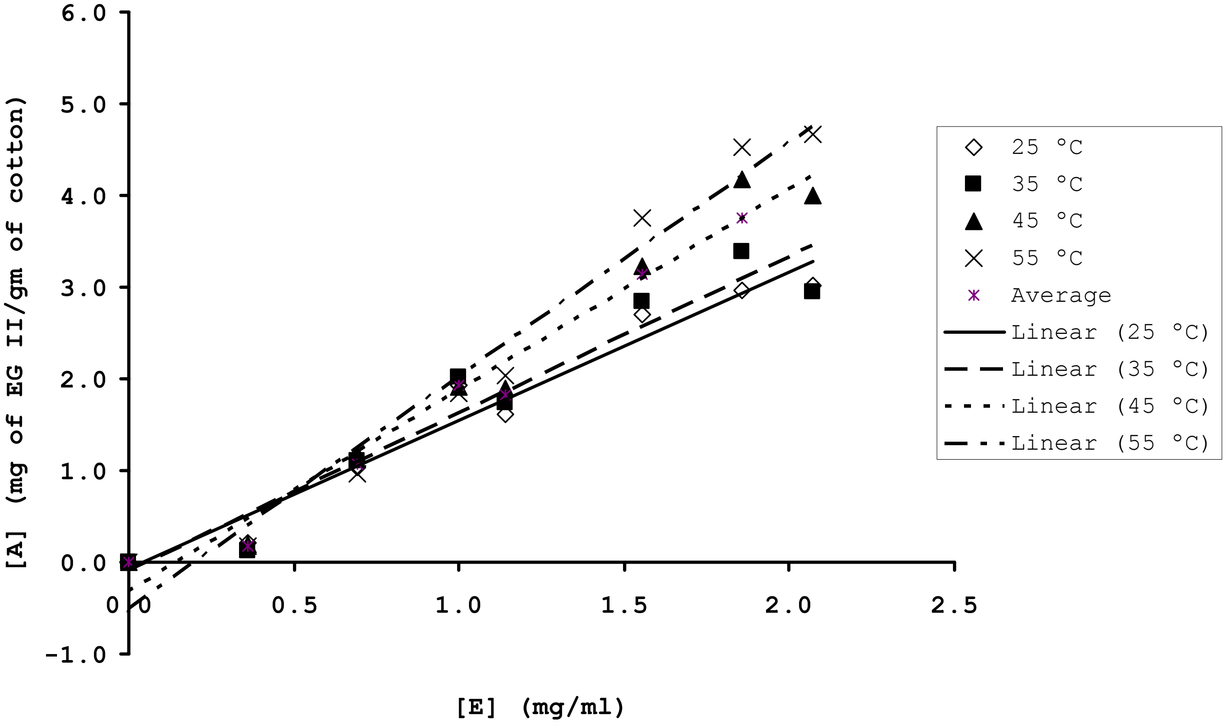

Figure 19. Linear plot for the adsorption isotherms of EG II on cotton fibers.

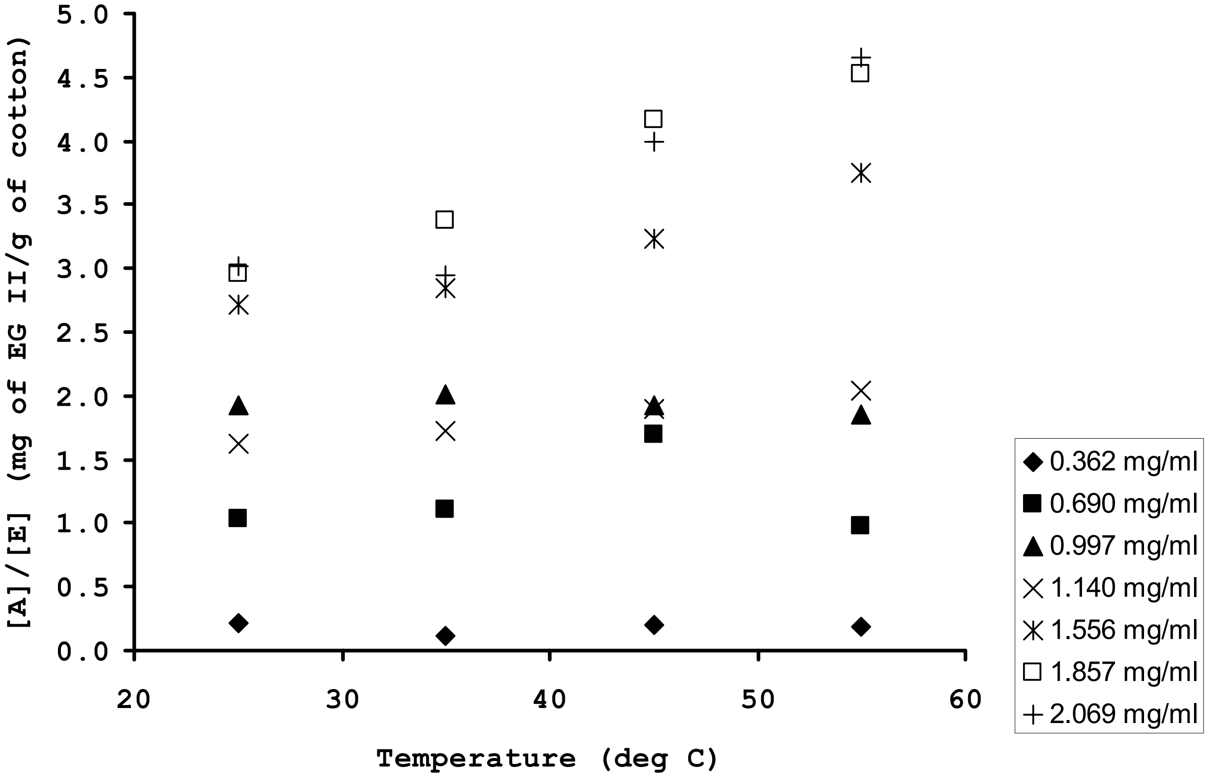

Figure 20. Effect of temperature on adsorption of EG II on cotton fibers at a given concentration of EG II

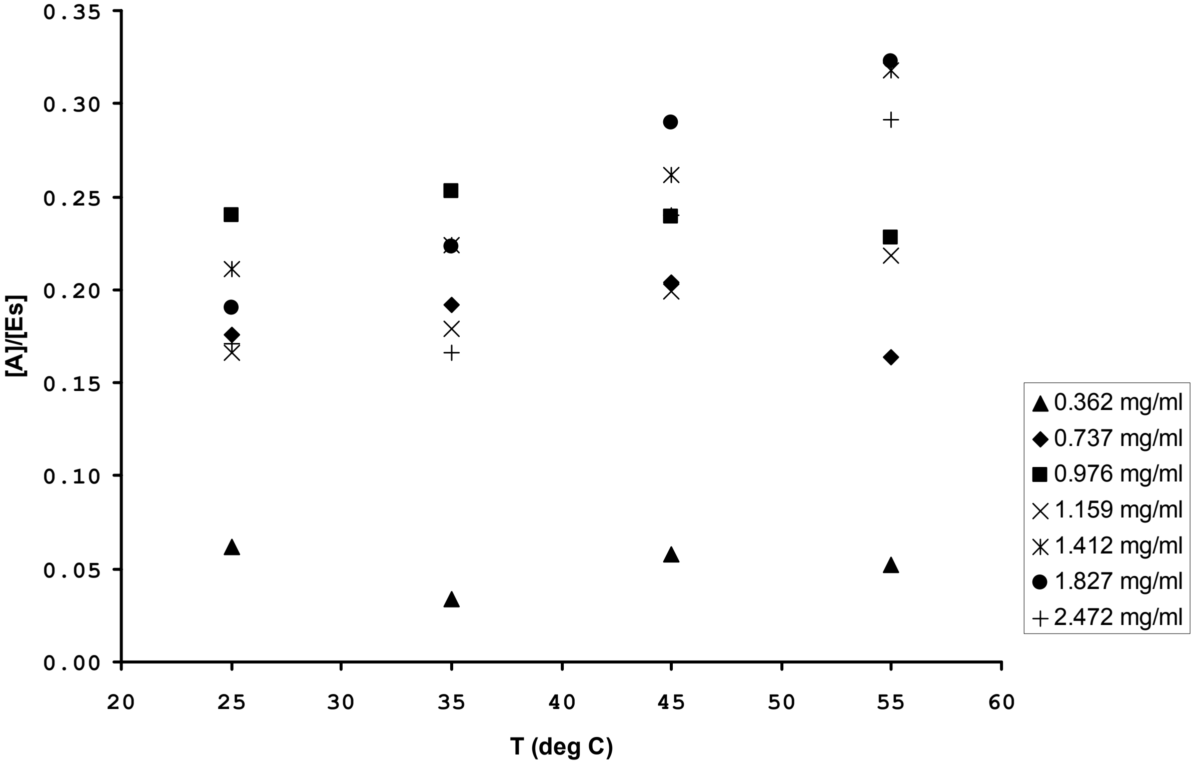

Figure 21. Effect of temperature on equilibrium adsorption of EG II on cotton fibers at a given concentration of EG II

concentrations against temperature of adsorption. From the figure 21 it is evident that the amount of EG II adsorbed on cotton fibers at equilibrium increases with the increase in temperature of adsorption. The increase in ratio, [A]/[Es] was higher at higher concentrations of EG II present in the adsorption bath.

Plot of [E] v/s [E]/[A]

Figure 22 is a plot of the amount of EG II present in solution against the ratio of the amount of EG II present in solution to the amount of EG II adsorbed per gram of cotton fibers. This plot used by Peitersen et.al. [74] and Kim et.al.[81] to model the adsorption of cellulases on microcrystalline cellulose was used to determine the goodness of fit of the Langmuir model for the adsorption of EG II on cotton.

Plot of 1/[E] v/s 1/[A]

Figure 23 is a plot of the inverse of the amount of EG II present in solution against the amount of EG II adsorbed per gram of cotton fibers. The plots of [E] against [E]/[A] and the reciprocal plots of [E] against [A] are the two methods employed to determine whether the experimental adsorption data can be explained by the Langmuir adsorption model.

Plot of ln[E] v/s ln[A]

Figure 24 is the natural logarithmic plot of the amount of EG II present in solution against the amount of EG II adsorbed

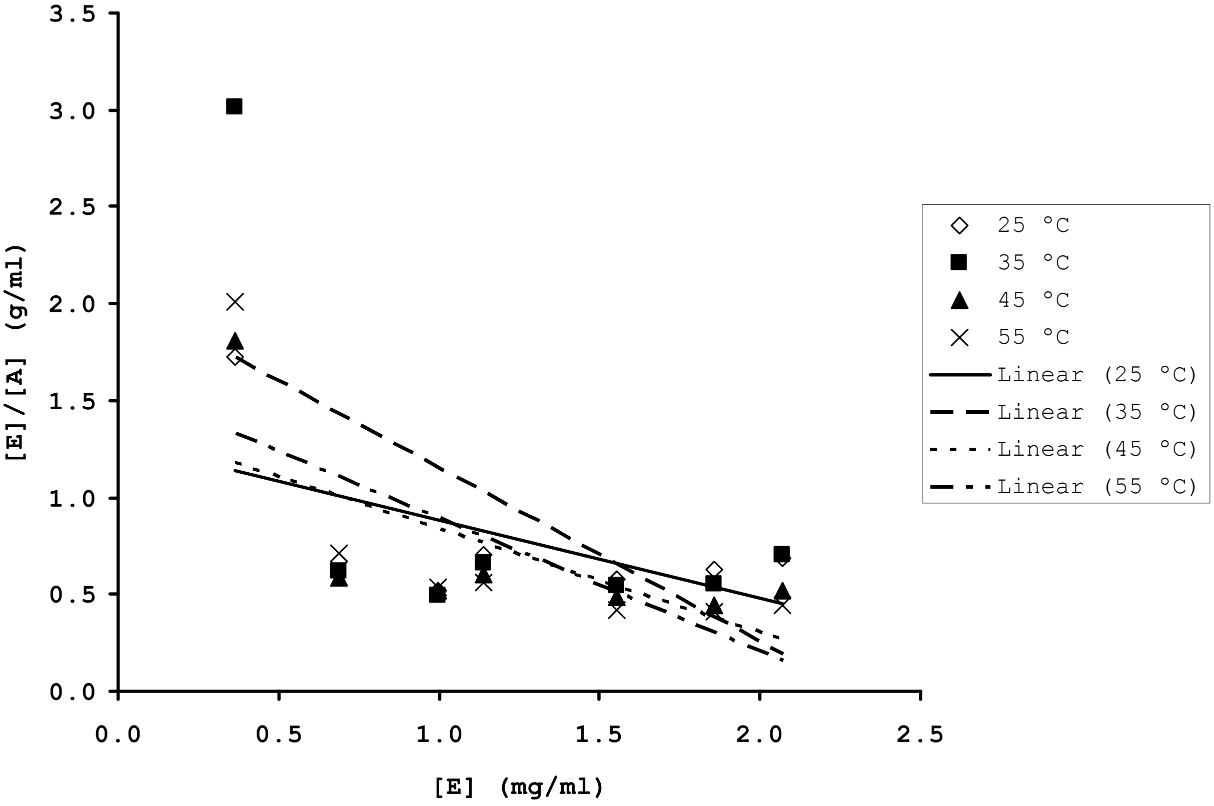

Figure 22. Langmuir plot for the adsorption isotherms of EG II on cotton fibers (Method I)

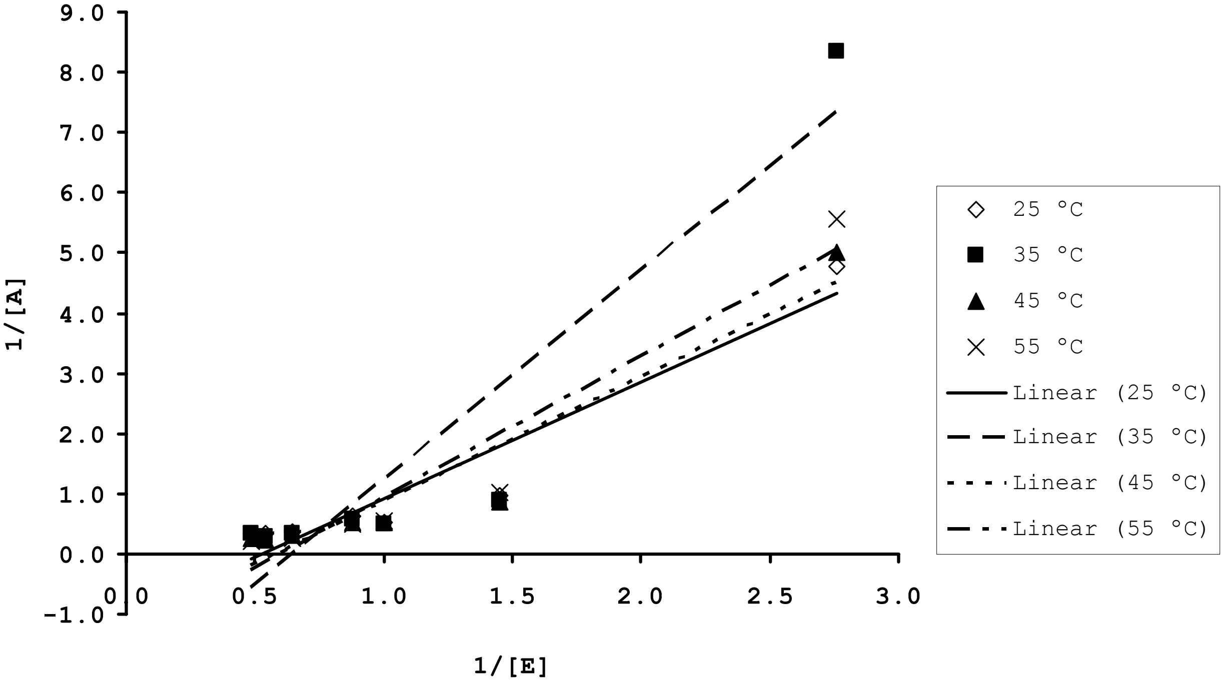

Figure 23. Langmuir plot for the adsorption isotherms of EG II on cotton fibers (Method II)

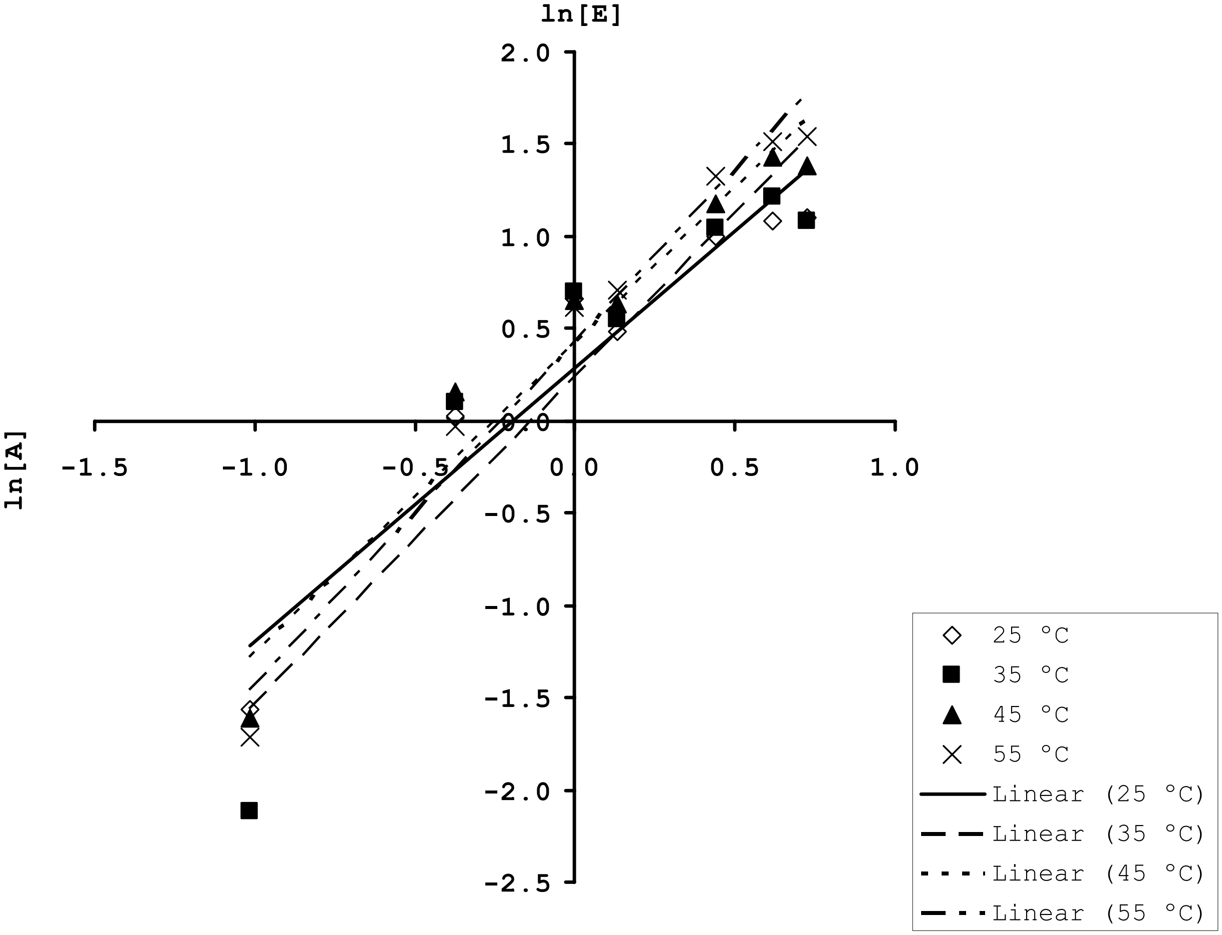

Figure 24. Freundlich plot for the adsorption isotherms of EG II on cotton fibers

per gram of cotton fibers. Plot of natural logarithms of [E] and [A] were made in order to determine whether the experimental adsorption data can be explained by Freundlich adsorption model. Plot of $1/[E]^{1/m}$ v/s 1/[A]

Figure 25 is the plot of inverse of the amount of EG II present in solution to the power of 1/m where m=2 against the inverse of amount of EG II adsorbed per gram of cotton fibers. The plot of $1/[E]^{1/m}$ v/s 1/[A] was made in order to determine whether the experimental adsorption data can be explained by the combined Langmuir-Freundlich adsorption model.

The R-square values of the linear regressions for the different models are listed in Table 6. The R-square values for the linear adsorption model were found to be greater than 0.9, ranging from 0.9329 to 0.9722. Values of R-square closer to 1 indicate that the linear adsorption model is a good fit of the experimental adsorption data for EG II.

The R-square values for the plot of [E] against [E]/[A] are found to be closer to 0 ranging from 0.3556 to 0.5461. From the R-square values, it may be said that the Langmuir adsorption model used by Peitersen et.al.[74] and Kim et.al. [81], may not be a good fit of the experimental adsorption data as per method I. But when the plots of the reciprocals of [E] and [A] were plotted, linear regression yielded R-square values that were closer to 1 indicating that Langmuir adsorption model

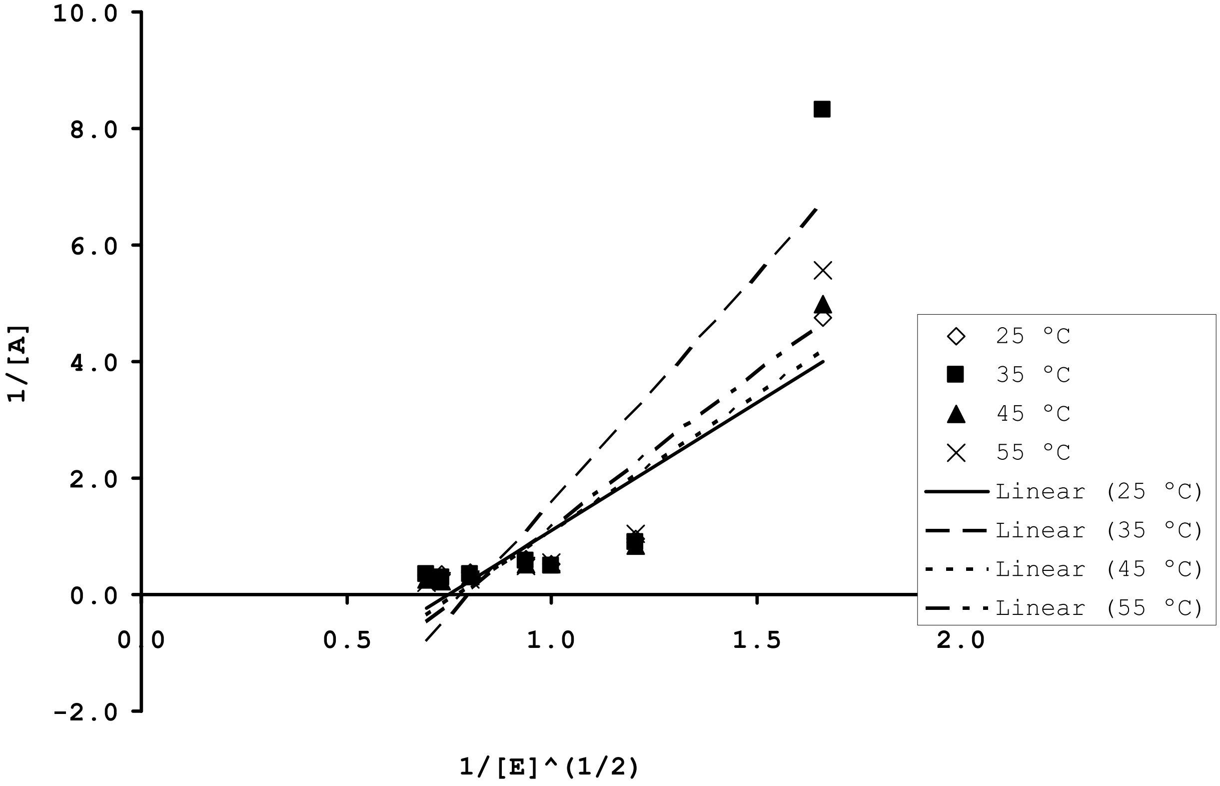

Figure 25. Combined Langmuir-Freundlich plot for the adsorption isotherms of EG II on cotton fibers

Table 6. R-square values for the different plots for the experimental adsorption data of EG II

Plot	**25 °C**	**35 °C**	**45 °C**	**55 °C**	**Average**
[E] v/s [A] (Linear)	0.9591	0.9329	0.9722	0.9697	0.9697
1/[E] v/s 1/[A] (Langmuir)	0.9164	0.8765	0.9112	0.9231	0.9078
[E] v/s [E]/[A] (Langmuir by Kim et.al.[81])	0.3589	0.3682	0.3556	0.5461	0.4430
Ln[E] v/s ln[A] (Freundlich)	0.9205	0.8735	0.9467	0.9719	0.9373
$1/[E]^{1/m}$ v/s 1/[A] Combined Langmuir-Freundlich)	0.8397	0.7892	0.8336	0.8482	0.8286

may be used for the experimental adsorption data as per Method II.

The R-square values for the Freundlich adsorption model obtained by the linear regression of the plot of natural logarithm of [E] against [A] were found to be close to 1 and were amongst highest R-square values for any model used to fit the adsorption data of EG II. Similarly, the R-square values for the combined Langmuir-Freundlich adsorption model were found to be close to 1 though the values were not as high as the R-square values for Freundlich adsorption model.

The R-square values for the average values of the adsorption of EG II at different temperatures at a given concentration are given in Table 6. The R-square values are all greater than 0.9 for all models except for the Langmuir model when tested by plotting [E] against [E]/[A]. For the combined Langmuir-Freundlich model the R-square value was found to be less than the R-square values for the other models.

The experimental adsorption data for CBH I, CBH II and EG II can be explained by more than one adsorption model. The range of enzyme concentrations used in the present study is not wide enough to determine whether a single adsorption model can explain the adsorption data. The determination of the enzyme concentration range used in this study was influenced by three factors:

1. The range of concentration of enzymes used in the adsorption experiments conducted by researchers on microcrystalline cellulose in the past studies[74] [81].
2. Limited quantities of pure enzymes available for the present work.
3. Practical range of concentration used in enzymatic processing of cotton fabrics with cellulases.

Thus, a typical enzyme concentration range used for enzymatic treatment of cotton may not be a very wide range to determine whether the adsorption data can be explained by a particular adsorption model. The enzyme concentration range in this research is similar to the range of enzyme concentration used in adsorption experiments conducted on microcrystalline cellulose by Kim et al [82] [83] to determine whether the Langmuir adsorption isotherm is a good fit for their adsorption data. Saturation of microcrystalline cellulose with the different enzymes used was observed in the study conducted by Kim et.al, [82] but in the present study, cotton was not saturated by the enzyme when treated by different purified enzymes in the given range of concentration of purified enzymes.

A possible explanation for the above mentioned observation may be more than those on cotton may be more as compared to the adsorption sites on microcrystalline cellulose. Cotton, which is highly crystalline, may have a large number of sites for adsorption of enzymes in the form or surface irregularities of crystal surfaces. With the hydrolysis of cotton, the irregularities on the surface of the crystal increase due to enzyme action and hence the adsorption sites for enzymes also increase. As a result of the increase in adsorption sites, the saturation of cotton fibers with the enzymes does not take place. Therefore, more than one models can explain the adsorption data of CBH I, CBH II and EG II.

Based on the structure of cellulases, the catalytic domain is responsible for the hydrolytic activity whereas the cellulose binding domain is responsible for the adsorption of cellulases on cellulose. Therefore, it may be possible that after inhibition of the enzyme by the cellulose hydrolysis product such as glucose or cellobiose the activity of purified enzymes would decrease but the enzyme may still get adsorbed resulting in non-hydrolytic adsorption of the enzyme.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

From the results of the experiments conducted in the present investigation, it is evident that experimental adsorption data for the adsorption of purified CBH I, CBH II and EG II on cotton fibers in this research can be explained by more than one adsorption model. The experimental adsorption data of purified CBH I, CBH II and EG II on cotton fibers obtained at different temperatures could be explained by the linear, Langmuir, Freundlich and combined Langmuir-Freundlich adsorption models.

The general concentration range of CBH I, CBH II and EG II used in the present investigation was in the range of 0.3 to 2.5 mg/ml which is the practical range of concentration of cellulases used in treatment of cotton with cellulases. The present range of concentration of purified enzymes was sufficient to saturate microcrystalline cellulose as observed in the studies reported [82] [74] but a similar range of concentration was not sufficient to saturate cotton fibers with enzymes. At this concentration range of enzymes, adsorption of purified enzymes was explained by more than one adsorption model, in the absence of saturation of the substrate with enzyme. Hence a wider range of enzyme concentration should to be used to determine the adsorption model that may best explain the adsorption of CBH I, II and EG II.

Effect of temperature on adsorption of enzymes

From figure 6, which is a plot of the adsorption of CBH I on cotton fibers at different adsorption temperatures at the different concentrations of CBH I studied, it can be said that the adsorption of CBH I on cotton fibers is affected the most at 55 °C. At lower temperatures, effect of temperature of adsorption is minimal in the range of concentration CBH I studied.

Similarly, when the adsorption of CBH II on cotton fibers was plotted against temperature at different concentrations as shown in figure 12, it was found that adsorption of CBH II is affected the most at 55 °C in the given range of concentration CBH II.

Adsorption of EG II on cotton fibers at low concentrations of EG II in the treatment bath was not affected by temperature of adsorption. At higher concentrations of EG II in the treatment bath studied, the amount of EG II adsorbed on cotton fibers increased slightly with increase in temperature of treatment.

Recommendations

Further research is recommended in the following areas:

1. Since the range of enzyme concentration used in the present study is not wide enough for a single adsorption model to explain the experimental adsorption data, higher concentrations of enzyme solutions are recommended in future studies. Though the range of concentration of purified enzymes used in the present study was enough to saturate microcrystalline cellulose, it was not enough to saturate cotton with the enzyme.
2. It is known that agitation of the treatment bath has a significant effect on the extent of cellulose hydrolysis. The effect of agitation on the adsorption of purified cellulases on cotton fibers may be investigated. Such a study may provide some valuable insight on the proven effect of agitation of treatment bath on cellulose hydrolysis.
3. When a mixture of two different enzymes was used in adsorption studied conducted on microcrystalline cellulose, the adsorption of one enzyme is influenced by the presence of the other enzyme in the mixture. Hence adsorption patterns of different mixtures of purified enzymes on cotton fibers such as CBH I and EG II, CBH II and EG II and CBH I and CBH II may be studied.

4. Pretreatment of the cotton fibers such as alkali treatment may influence the adsorption of purified cellulases on cotton fibers. Hence it may be of interest to study the adsorption pattern of purified cellulases on alkali treated cotton fibers.
5. The ionic strength of the enzymatic solution has been said to influence the adsorption pattern of purified cellulases on microcrystalline cellulose [80]. The effect of additives in enzymatic treatment of cotton such as wetting agents and salts present in the buffer system may also affect the adsorption pattern of purified cellulases on cotton.

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Appendix A

Statistical Analysis for Experimental Adsorption Data of CBH I to fit Adsorption Models

SAS CODE

```
%let path=D:\Dole Files\;
libname bus "&path.";
/* THIS SAS FILE IS FOR REGRESSION FOR DATA ON CBH1a FILE */

/* This step makes new variables for calculating different models */

data temp;
set bus.CBHIa;
m=2;
if E>0.001 then do;
EbyA=E/A;
Einv=1/E;
Ainv=1/A;
LNE=log(E);
LNA=log(A);
CMBLNGE=1/(E**(1/m));
drop m;
end;
run;

/**********************************************/
/* regression models ..... FOR TEMPERATURE=25 */
/**********************************************/

/* Selecting Cases */

data temp25;
set temp;
if TEMP=25 then output;
run;

/* A versus E Regression */
title 'A versus E REGRESSION on TEMPERATURE=25';
PROC REG data=temp25;
  MODEL A=E;
OUTPUT OUT=AE P=PRED R=RESID;
PLOT A*E='*' PREDICTED.*E='o' RESIDUAL.*E='x';
PROC PRINT DATA=AE;
run;

/* EbyA versus E Regression */
title 'EbyA versus E REGRESSION on TEMPERATURE=25';
PROC REG data=temp25;
  MODEL EbyA=E;
OUTPUT OUT=EbyA P=PRED R=RESID;
PLOT AbyE*E='*' PREDICTED.*E='o' RESIDUAL.*E='x';
PROC PRINT DATA=EbyA;
run;

/* Langmuir Regression */
title 'LANGMUIR REGRESSION on TEMPERATURE=25';
PROC REG data=temp25;
  MODEL Ainv=Einv;
OUTPUT OUT=LANGMUIR P=PRED R=RESID;
PLOT Ainv*Einv='*' PREDICTED.*Einv='o' RESIDUAL.*Einv='x';
```

```
PROC PRINT DATA=LANGMUIR;
run;

/* Freundlich Regression */
title 'FREUNDLICH REGRESSION on TEMPERATURE=25';
PROC REG data=temp25;
MODEL LNA=LNE;
OUTPUT OUT=FREUNDLICH P=PRED R=RESID;
PLOT LNA*LNE='*' PREDICTED.*LNE='o' RESIDUAL.*LNE='x';
PROC PRINT DATA=FREUNDLICH;
run;

/* Combined Langmuir Regression */
title 'COMBINED LANGMUIR REGRESSION on TEMPERATURE=25';
PROC REG data=temp25;
MODEL Ainv=CMBLNGE;
OUTPUT OUT=COMBLANGMUIR P=PRED R=RESID;
PLOT Ainv*CMBLNGE='*' PREDICTED.*CMBLNGE='o' RESIDUAL.*CMBLNGE='x';
PROC PRINT DATA=COMBLANGMUIR;
run;



/**********************************************/
/* regression models ..... FOR TEMPERATURE=35 */
/**********************************************/

/* Selecting Cases */

data temp35;
set temp;
if TEMP=35 then output;
run;

/* A versus E Regression */
title 'A versus E REGRESSION on TEMPERATURE=35';
PROC REG data=temp35;
  MODEL A=E;
OUTPUT OUT=AE P=PRED R=RESID;
PLOT A*E='*' PREDICTED.*E='o' RESIDUAL.*E='x';
PROC PRINT DATA=AE;
run;

/* EbyA versus E Regression */
title 'EbyA versus E REGRESSION on TEMPERATURE=35';
PROC REG data=temp35;
  MODEL EbyA=E;
OUTPUT OUT=EbyA P=PRED R=RESID;
PLOT EbyA*E='*' PREDICTED.*E='o' RESIDUAL.*E='x';
PROC PRINT DATA=EbyA;
run;

/* Langmuir Regression */
title 'LANGMUIR REGRESSION on TEMPERATURE=35';
PROC REG data=temp35;
  MODEL Ainv=Einv;
OUTPUT OUT=LANGMUIR P=PRED R=RESID;
```

```
PLOT Ainv*Einv='*' PREDICTED.*Einv='o' RESIDUAL.*Einv='x';
PROC PRINT DATA=LANGMUIR;
run;

/* Freundlich Regression */
title 'FREUNDLICH REGRESSION on TEMPERATURE=35';
PROC REG data=temp35;
MODEL LNA=LNE;
OUTPUT OUT=FREUNDLICH P=PRED R=RESID;
PLOT LNA*LNE='*' PREDICTED.*LNE='o' RESIDUAL.*LNE='x';
PROC PRINT DATA=FREUNDLICH;
run;

/* Combined Langmuir Regression */
title 'COMBINED LANGMUIR REGRESSION on TEMPERATURE=35';
PROC REG data=temp35;
MODEL Ainv=CMBLNGE;
OUTPUT OUT=COMBLANGMUIR P=PRED R=RESID;
PLOT Ainv*CMBLNGE='*' PREDICTED.*CMBLNGE='o' RESIDUAL.*CMBLNGE='x';
PROC PRINT DATA=COMBLANGMUIR;
run;



/**********************************************/
/* regression models ..... FOR TEMPERATURE=45 */
/**********************************************/

/* Selecting Cases */

data temp45;
set temp;
if TEMP=45 then output;
run;

/* A versus E Regression */
title 'A versus E REGRESSION on TEMPERATURE=45';
PROC REG data=temp45;
  MODEL A=E;
OUTPUT OUT=AE P=PRED R=RESID;
PLOT A*E='*' PREDICTED.*E='o' RESIDUAL.*E='x';
PROC PRINT DATA=AE;
run;

/* EbyA versus E Regression */
title 'EbyA versus E REGRESSION on TEMPERATURE=45';
PROC REG data=temp45;
  MODEL EbyA=E;
OUTPUT OUT=EbyA P=PRED R=RESID;
PLOT EbyA*E='*' PREDICTED.*E='o' RESIDUAL.*E='x';
PROC PRINT DATA=EbyA;
run;

/* Langmuir Regression */
title 'LANGMUIR REGRESSION on TEMPERATURE=45';
PROC REG data=temp45;
  MODEL Ainv=Einv;
```

```
OUTPUT OUT=LANGMUIR P=PRED R=RESID;
PLOT Ainv*Einv='*' PREDICTED.*Einv='o' RESIDUAL.*Einv='x';
PROC PRINT DATA=LANGMUIR;
run;

/* Freundlich Regression */
title 'FREUNDLICH REGRESSION on TEMPERATURE=45';
PROC REG data=temp45;
MODEL LNA=LNE;
OUTPUT OUT=FREUNDLICH P=PRED R=RESID;
PLOT LNA*LNE='*' PREDICTED.*LNE='o' RESIDUAL.*LNE='x';
PROC PRINT DATA=FREUNDLICH;
run;

/* Combined Langmuir Regression */
title 'COMBINED LANGMUIR REGRESSION on TEMPERATURE=45';
PROC REG data=temp45;
MODEL Ainv=CMBLNGE;
OUTPUT OUT=COMBLANGMUIR P=PRED R=RESID;
PLOT Ainv*CMBLNGE='*' PREDICTED.*CMBLNGE='o' RESIDUAL.*CMBLNGE='x';
PROC PRINT DATA=COMBLANGMUIR;
run;


/**********************************************/
/* regression models ..... FOR TEMPERATURE=55 */
/**********************************************/

/* Selecting Cases */

data temp55;
set temp;
if TEMP=55 then output;
run;

/* A versus E Regression */
title 'A versus E REGRESSION on TEMPERATURE=55';
PROC REG data=temp55;
  MODEL A=E;
OUTPUT OUT=AE P=PRED R=RESID;
PLOT A*E='*' PREDICTED.*E='o' RESIDUAL.*E='x';
PROC PRINT DATA=AE;
run;

/* EbyA versus E Regression */
title 'EbyA versus E REGRESSION on TEMPERATURE=55';
PROC REG data=temp55;
  MODEL EbyA=E;
OUTPUT OUT=EbyA P=PRED R=RESID;
PLOT EbyA*E='*' PREDICTED.*E='o' RESIDUAL.*E='x';
PROC PRINT DATA=EbyA;
run;

/* Langmuir Regression */
title 'LANGMUIR REGRESSION on TEMPERATURE=55';
PROC REG data=temp55;
  MODEL Ainv=Einv;
```

```
OUTPUT OUT=LANGMUIR P=PRED R=RESID;
PLOT Ainv*Einv='*' PREDICTED.*Einv='o' RESIDUAL.*Einv='x';
PROC PRINT DATA=LANGMUIR;
run;

/* Freundlich Regression */
title 'FREUNDLICH REGRESSION on TEMPERATURE=55';
PROC REG data=temp55;
MODEL LNA=LNE;
OUTPUT OUT=FREUNDLICH P=PRED R=RESID;
PLOT LNA*LNE='*' PREDICTED.*LNE='o' RESIDUAL.*LNE='x';
PROC PRINT DATA=FREUNDLICH;
run;

/* Combined Langmuir Regression */
title 'COMBINED LANGMUIR REGRESSION on TEMPERATURE=55';
PROC REG data=temp55;
MODEL Ainv=CMBLNGE;
OUTPUT OUT=COMBLANGMUIR P=PRED R=RESID;
PLOT Ainv*CMBLNGE='*' PREDICTED.*CMBLNGE='o' RESIDUAL.*CMBLNGE='x';
PROC PRINT DATA=COMBLANGMUIR;
run;
```

SAS OUTPUT

```
                            A versus E REGRESSION on TEMPERATURE=25
1
                                                                18:08 Saturday, October
25, 2003

                                       The REG Procedure
                                         Model: MODEL1
                                     Dependent Variable: A

                                      Analysis of Variance

                                             Sum of           Mean
         Source                   DF        Squares         Square    F Value    Pr > F

         Model                     1        8.59412        8.59412      97.82    <.0001
         Error                     6        0.52714        0.08786
         Corrected Total           7        9.12126


                      Root MSE              0.29641    R-Square     0.9422
                      Dependent Mean        1.33963    Adj R-Sq     0.9326
                      Coeff Var            22.12601


                                      Parameter Estimates

                                   Parameter       Standard
              Variable     DF       Estimate          Error    t Value    Pr > |t|

              Intercept     1       -0.27850        0.19429      -1.43      0.2017
              E             1        1.42613        0.14419       9.89      <.0001

                            A versus E REGRESSION on TEMPERATURE=25
2
                                                                18:08 Saturday, October
25, 2003

           Obs            Temp              E              A      EbyA       Einv

            1               25          0.001          0.001     .          .
            2               25          0.493           0.39    1.26410    2.02840
            3               25          0.737           0.61    1.20820    1.35685
            4               25          0.976            0.9    1.08444    1.02459
            5               25          1.159           0.99    1.17071    0.86281
            6               25          1.412           1.94    0.72784    0.70822
            7               25          1.827          2.746    0.66533    0.54735
            8               25          2.472           3.14    0.78726    0.40453

           Obs      Ainv        LNE         LNA      CMBLNGE      PRED         RESID

            1      .           .           .          .         -0.27707     0.27807
            2     2.56410    -0.70725    -0.94161    1.42422     0.42458    -0.03458
            3     1.63934    -0.30517    -0.49430    1.16484     0.77256    -0.16256
            4     1.11111    -0.02429    -0.10536    1.01222     1.11340    -0.21340
            5     1.01010     0.14756    -0.01005    0.92888     1.37439    -0.38439
            6     0.51546     0.34501     0.66269    0.84156     1.73520     0.20480
            7     0.36417     0.60268     1.01015    0.73983     2.32704     0.41896
            8     0.31847     0.90503     1.14422    0.63603     3.24690    -0.10690

                           EbyA versus E REGRESSION on TEMPERATURE=25
3
                                                                18:08 Saturday, October
25, 2003

                                       The REG Procedure
                                         Model: MODEL1
                                   Dependent Variable: EbyA
```

```
                                      Analysis of Variance

                                             Sum of           Mean
         Source                   DF        Squares         Square    F Value    Pr > F

         Model                     1        0.25489        0.25489      10.23    0.0240
         Error                     5        0.12460        0.02492
         Corrected Total           6        0.37949


                      Root MSE              0.15786    R-Square     0.6717
                      Dependent Mean        0.98684    Adj R-Sq     0.6060
                      Coeff Var            15.99637


                                      Parameter Estimates

                                   Parameter       Standard
              Variable     DF       Estimate          Error    t Value    Pr > |t|

              Intercept     1        1.38109        0.13695      10.08      0.0002
              E             1       -0.30407        0.09507      -3.20      0.0240

                           EbyA versus E REGRESSION on TEMPERATURE=25
4
                                                                18:08 Saturday, October
25, 2003

           Obs           Temp              E              A      EbyA       Einv

            1              25          0.001          0.001       .          .
            2              25          0.493           0.39    1.26410    2.02840
            3              25          0.737           0.61    1.20820    1.35685
            4              25          0.976            0.9    1.08444    1.02459
            5              25          1.159           0.99    1.17071    0.86281
            6              25          1.412           1.94    0.72784    0.70822
            7              25          1.827          2.746    0.66533    0.54735
            8              25          2.472           3.14    0.78726    0.40453

           Obs      Ainv        LNE         LNA      CMBLNGE      PRED       RESID

            1      .           .           .          .         1.38078      .
            2     2.56410    -0.70725    -0.94161    1.42422    1.23118     0.03292
            3     1.63934    -0.30517    -0.49430    1.16484    1.15699     0.05121
            4     1.11111    -0.02429    -0.10536    1.01222    1.08432     0.00013
            5     1.01010     0.14756    -0.01005    0.92888    1.02867     0.14204
            6     0.51546     0.34501     0.66269    0.84156    0.95174    -0.22391
            7     0.36417     0.60268     1.01015    0.73983    0.82555    -0.16022
            8     0.31847     0.90503     1.14422    0.63603    0.62943     0.15783

                             LANGMUIR REGRESSION on TEMPERATURE=25
5
                                                                18:08 Saturday, October
25, 2003

                                       The REG Procedure
                                         Model: MODEL1
                                   Dependent Variable: Ainv

                                      Analysis of Variance

                                             Sum of           Mean
         Source                   DF        Squares         Square    F Value    Pr > F

         Model                     1        3.88024        3.88024     373.95    <.0001
         Error                     5        0.05188        0.01038
         Corrected Total           6        3.93213


                      Root MSE              0.10187    R-Square     0.9868
                      Dependent Mean        1.07468    Adj R-Sq     0.9842
```

```
                      Coeff Var            9.47864


                                      Parameter Estimates

                                   Parameter       Standard
              Variable     DF       Estimate          Error    t Value    Pr > |t|

              Intercept     1       -0.36030        0.08360      -4.31      0.0076
              Einv          1        1.44890        0.07493      19.34      <.0001

                             LANGMUIR REGRESSION on TEMPERATURE=25
6
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25, 2003

           Obs            Temp                E               A      EbyA       Einv

            1               25            0.001           0.001        .          .
            2               25            0.493            0.39    1.26410    2.02840
            3               25            0.737            0.61    1.20820    1.35685
            4               25            0.976             0.9    1.08444    1.02459
            5               25            1.159            0.99    1.17071    0.86281
            6               25            1.412            1.94    0.72784    0.70822
            7               25            1.827           2.746    0.66533    0.54735
            8               25            2.472            3.14    0.78726    0.40453

           Obs      Ainv        LNE         LNA      CMBLNGE      PRED       RESID

            1        .           .           .          .          .           .
            2     2.56410    -0.70725    -0.94161    1.42422    2.57865    -0.01454
            3     1.63934    -0.30517    -0.49430    1.16484    1.60564     0.03370
            4     1.11111    -0.02429    -0.10536    1.01222    1.12423    -0.01312
            5     1.01010     0.14756    -0.01005    0.92888    0.88983     0.12027
            6     0.51546     0.34501     0.66269    0.84156    0.66583    -0.15037
            7     0.36417     0.60268     1.01015    0.73983    0.43275    -0.06858
            8     0.31847     0.90503     1.14422    0.63603    0.22583     0.09265

                            FREUNDLICH REGRESSION on TEMPERATURE=25
7
                                                                18:08 Saturday, October
25, 2003

                                       The REG Procedure
                                         Model: MODEL1
                                    Dependent Variable: LNA

                                      Analysis of Variance

                                            Sum of           Mean
         Source                  DF        Squares         Square    F Value    Pr > F

         Model                    1        3.54933        3.54933     133.69    <.0001
         Error                    5        0.13275        0.02655
         Corrected Total          6        3.68208


                      Root MSE             0.16294    R-Square     0.9639
                      Dependent Mean       0.18082    Adj R-Sq     0.9567
                      Coeff Var           90.11203


                                      Parameter Estimates

                                   Parameter       Standard
              Variable     DF       Estimate          Error    t Value    Pr > |t|

              Intercept     1       -0.01332        0.06383      -0.21      0.8430
              LNE           1        1.41036        0.12198      11.56      <.0001

                            FREUNDLICH REGRESSION on TEMPERATURE=25
```

```
8
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          Obs           Temp              E               A      EbyA       Einv

            1             25          0.001           0.001        .          .
            2             25          0.493            0.39    1.26410    2.02840
            3             25          0.737            0.61    1.20820    1.35685
            4             25          0.976             0.9    1.08444    1.02459
            5             25          1.159            0.99    1.17071    0.86281
            6             25          1.412            1.94    0.72784    0.70822
            7             25          1.827           2.746    0.66533    0.54735
            8             25          2.472            3.14    0.78726    0.40453

          Obs      Ainv        LNE         LNA      CMBLNGE      PRED         RESID

            1        .          .           .          .          .            .
            2     2.56410    -0.70725    -0.94161    1.42422    -1.01079     0.06918
            3     1.63934    -0.30517    -0.49430    1.16484    -0.44371    -0.05058
            4     1.11111    -0.02429    -0.10536    1.01222    -0.04758    -0.05778
            5     1.01010     0.14756    -0.01005    0.92888     0.19479    -0.20484
            6     0.51546     0.34501     0.66269    0.84156     0.47327     0.18942
            7     0.36417     0.60268     1.01015    0.73983     0.83667     0.17347
            8     0.31847     0.90503     1.14422    0.63603     1.26310    -0.11887

                     COMBINED LANGMUIR REGRESSION on TEMPERATURE=25
9
                                                     18:08 Saturday, October
25, 2003

                                   The REG Procedure
                                     Model: MODEL1
                                Dependent Variable: Ainv

                                  Analysis of Variance

                                         Sum of          Mean
        Source                   DF        Squares        Square    F Value    Pr > F

        Model                     1        3.81548       3.81548     163.55    <.0001
        Error                     5        0.11664       0.02333
        Corrected Total           6        3.93213


                     Root MSE              0.15274    R-Square     0.9703
                     Dependent Mean        1.07468    Adj R-Sq     0.9644
                     Coeff Var            14.21244


                                  Parameter Estimates

                                Parameter       Standard
             Variable     DF       Estimate          Error    t Value    Pr > |t|

             Intercept     1       -1.80169        0.23221      -7.76      0.0006
             CMBLNGE       1        2.98397        0.23333      12.79      <.0001

                     COMBINED LANGMUIR REGRESSION on TEMPERATURE=25
10
                                                     18:08 Saturday, October
25, 2003

          Obs           Temp              E               A      EbyA       Einv

            1             25          0.001           0.001        .          .
            2             25          0.493            0.39    1.26410    2.02840
            3             25          0.737            0.61    1.20820    1.35685
            4             25          0.976             0.9    1.08444    1.02459
            5             25          1.159            0.99    1.17071    0.86281
            6             25          1.412            1.94    0.72784    0.70822
```

```
            7              25           1.827           2.746    0.66533    0.54735
            8              25           2.472            3.14    0.78726    0.40453

          Obs      Ainv         LNE          LNA      CMBLNGE       PRED       RESID

            1        .           .            .          .           .            .
            2     2.56410    -0.70725    -0.94161    1.42422    2.44814     0.11596
            3     1.63934    -0.30517    -0.49430    1.16484    1.67417    -0.03482
            4     1.11111    -0.02429    -0.10536    1.01222    1.21875    -0.10764
            5     1.01010     0.14756    -0.01005    0.92888    0.97006     0.04004
            6     0.51546     0.34501     0.66269    0.84156    0.70949    -0.19403
            7     0.36417     0.60268     1.01015    0.73983    0.40594    -0.04178
            8     0.31847     0.90503     1.14422    0.63603    0.09620     0.22227

                            A versus E REGRESSION on TEMPERATURE=35
11
                                                               18:08 Saturday, October
25, 2003

                                         The REG Procedure
                                           Model: MODEL1
                                       Dependent Variable: A

                                        Analysis of Variance

                                              Sum of           Mean
         Source                   DF         Squares         Square    F Value    Pr > F

         Model                     1         8.03863        8.03863      45.24    0.0005
         Error                     6         1.06609        0.17768
         Corrected Total           7         9.10472


                      Root MSE              0.42152    R-Square     0.8829
                      Dependent Mean        1.43375    Adj R-Sq     0.8634
                      Coeff Var            29.40006


                                        Parameter Estimates

                                    Parameter       Standard
              Variable     DF        Estimate          Error    t Value    Pr > |t|

              Intercept     1        -0.13121        0.27630      -0.47      0.6517
              E             1         1.37927        0.20506       6.73      0.0005

                            A versus E REGRESSION on TEMPERATURE=35
12
                                                               18:08 Saturday, October
25, 2003

          Obs           Temp              E              A      EbyA       Einv

            1             35          0.001          0.001        .           .
            2             35          0.493           0.45    1.09556    2.02840
            3             35          0.737           0.44    1.67500    1.35685
            4             35          0.976           0.83    1.17590    1.02459
            5             35          1.159           1.81    0.64033    0.86281
            6             35          1.412          2.516    0.56121    0.70822
            7             35          1.827          2.434    0.75062    0.54735
            8             35          2.472          2.989    0.82703    0.40453

          Obs      Ainv         LNE          LNA      CMBLNGE       PRED         RESID

            1        .           .            .          .        -0.12983     0.13083
            2     2.22222    -0.70725    -0.79851    1.42422     0.54877    -0.09877
            3     2.27273    -0.30517    -0.82098    1.16484     0.88532    -0.44532
            4     1.20482    -0.02429    -0.18633    1.01222     1.21496    -0.38496
            5     0.55249     0.14756     0.59333    0.92888     1.46737     0.34263
            6     0.39746     0.34501     0.92267    0.84156     1.81633     0.69967
            7     0.41085     0.60268     0.88954    0.73983     2.38872     0.04528
```

```
          8     0.33456     0.90503     1.09494    0.63603     3.27835    -0.28935

                      EbyA versus E REGRESSION on TEMPERATURE=35
13
                                                           18:08 Saturday, October
25, 2003

                                  The REG Procedure
                                    Model: MODEL1
                                Dependent Variable: EbyA

                                 Analysis of Variance

                                        Sum of           Mean
      Source                  DF       Squares         Square    F Value    Pr > F

      Model                    1       0.26063        0.26063       2.04    0.2124
      Error                    5       0.63833        0.12767
      Corrected Total          6       0.89895


                   Root MSE             0.35730    R-Square     0.2899
                   Dependent Mean       0.96081    Adj R-Sq     0.1479
                   Coeff Var           37.18788


                                 Parameter Estimates

                                Parameter       Standard
           Variable     DF       Estimate          Error    t Value    Pr > |t|

           Intercept     1        1.35946        0.30998       4.39      0.0071
           E             1       -0.30747        0.21519      -1.43      0.2124

                      EbyA versus E REGRESSION on TEMPERATURE=35
14
                                                           18:08 Saturday, October
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          Obs           Temp              E              A      EbyA       Einv

           1             35           0.001          0.001     .          .
           2             35           0.493           0.45    1.09556    2.02840
           3             35           0.737           0.44    1.67500    1.35685
           4             35           0.976           0.83    1.17590    1.02459
           5             35           1.159           1.81    0.64033    0.86281
           6             35           1.412          2.516    0.56121    0.70822
           7             35           1.827          2.434    0.75062    0.54735
           8             35           2.472          2.989    0.82703    0.40453

          Obs      Ainv        LNE         LNA      CMBLNGE      PRED       RESID

           1     .           .           .           .          1.35916     .
           2     2.22222    -0.70725    -0.79851    1.42422    1.20788    -0.11232
           3     2.27273    -0.30517    -0.82098    1.16484    1.13286     0.54214
           4     1.20482    -0.02429    -0.18633    1.01222    1.05937     0.11653
           5     0.55249     0.14756     0.59333    0.92888    1.00311    -0.36277
           6     0.39746     0.34501     0.92267    0.84156    0.92532    -0.36411
           7     0.41085     0.60268     0.88954    0.73983    0.79772    -0.04710
           8     0.33456     0.90503     1.09494    0.63603    0.59940     0.22763

                        LANGMUIR REGRESSION on TEMPERATURE=35
15
                                                           18:08 Saturday, October
25, 2003

                                  The REG Procedure
                                    Model: MODEL1
                                Dependent Variable: Ainv

                                 Analysis of Variance
```

```
                                    Sum of           Mean
Source                   DF        Squares         Square    F Value    Pr > F

Model                     1        3.71403        3.71403      24.04    0.0045
Error                     5        0.77252        0.15450
Corrected Total           6        4.48655


             Root MSE              0.39307    R-Square     0.8278
             Dependent Mean        1.05645    Adj R-Sq     0.7934
             Coeff Var            37.20677


                              Parameter Estimates

                          Parameter       Standard
     Variable     DF       Estimate          Error    t Value    Pr > |t|

     Intercept     1       -0.34746        0.32259      -1.08      0.3306
     Einv          1        1.41753        0.28912       4.90      0.0045

                     LANGMUIR REGRESSION on TEMPERATURE=35
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25, 2003

  Obs           Temp              E              A      EbyA       Einv

   1             35           0.001          0.001       .          .
   2             35           0.493           0.45    1.09556    2.02840
   3             35           0.737           0.44    1.67500    1.35685
   4             35           0.976           0.83    1.17590    1.02459
   5             35           1.159           1.81    0.64033    0.86281
   6             35           1.412          2.516    0.56121    0.70822
   7             35           1.827          2.434    0.75062    0.54735
   8             35           2.472          2.989    0.82703    0.40453

  Obs      Ainv        LNE          LNA      CMBLNGE      PRED       RESID

   1        .           .            .          .          .           .
   2     2.22222    -0.70725    -0.79851    1.42422    2.52785    -0.30563
   3     2.27273    -0.30517    -0.82098    1.16484    1.57591     0.69681
   4     1.20482    -0.02429    -0.18633    1.01222    1.10492     0.09990
   5     0.55249     0.14756     0.59333    0.92888    0.87560    -0.32311
   6     0.39746     0.34501     0.92267    0.84156    0.65645    -0.25900
   7     0.41085     0.60268     0.88954    0.73983    0.42841    -0.01757
   8     0.33456     0.90503     1.09494    0.63603    0.22597     0.10859

                    FREUNDLICH REGRESSION on TEMPERATURE=35
17
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                               The REG Procedure
                                 Model: MODEL1
                            Dependent Variable: LNA

                              Analysis of Variance

                                    Sum of           Mean
Source                   DF        Squares         Square    F Value    Pr > F

Model                     1        3.55737        3.55737      31.08    0.0026
Error                     5        0.57223        0.11445
Corrected Total           6        4.12960


             Root MSE              0.33830    R-Square     0.8614
             Dependent Mean        0.24209    Adj R-Sq     0.8337
             Coeff Var           139.73843
```

```
                                      Parameter Estimates

                                   Parameter       Standard
              Variable     DF       Estimate          Error    t Value    Pr > |t|

              Intercept     1        0.04774        0.13253       0.36      0.7334
              LNE           1        1.41196        0.25325       5.58      0.0026

                            FREUNDLICH REGRESSION on TEMPERATURE=35
18
                                                                18:08 Saturday, October
25, 2003

           Obs           Temp              E               A      EbyA       Einv

            1             35           0.001           0.001     .          .
            2             35           0.493            0.45    1.09556    2.02840
            3             35           0.737            0.44    1.67500    1.35685
            4             35           0.976            0.83    1.17590    1.02459
            5             35           1.159            1.81    0.64033    0.86281
            6             35           1.412           2.516    0.56121    0.70822
            7             35           1.827           2.434    0.75062    0.54735
            8             35           2.472           2.989    0.82703    0.40453

           Obs      Ainv        LNE         LNA      CMBLNGE      PRED         RESID

            1      .          .           .          .           .            .
            2     2.22222    -0.70725    -0.79851    1.42422    -0.95087     0.15236
            3     2.27273    -0.30517    -0.82098    1.16484    -0.38315    -0.43783
            4     1.20482    -0.02429    -0.18633    1.01222     0.01343    -0.19976
            5     0.55249     0.14756     0.59333    0.92888     0.25608     0.33725
            6     0.39746     0.34501     0.92267    0.84156     0.53487     0.38780
            7     0.41085     0.60268     0.88954    0.73983     0.89869    -0.00915
            8     0.33456     0.90503     1.09494    0.63603     1.32560    -0.23066

                         COMBINED LANGMUIR REGRESSION on TEMPERATURE=35
19
                                                                18:08 Saturday, October
25, 2003

                                       The REG Procedure
                                         Model: MODEL1
                                   Dependent Variable: Ainv

                                      Analysis of Variance

                                             Sum of           Mean
         Source                  DF         Squares         Square    F Value    Pr > F

         Model                    1         3.75107        3.75107      25.50    0.0039
         Error                    5         0.73548        0.14710
         Corrected Total          6         4.48655


                      Root MSE              0.38353    R-Square     0.8361
                      Dependent Mean        1.05645    Adj R-Sq     0.8033
                      Coeff Var            36.30394


                                      Parameter Estimates

                                   Parameter       Standard
              Variable     DF       Estimate          Error    t Value    Pr > |t|

              Intercept     1       -1.79554        0.58308      -3.08      0.0275
              CMBLNGE       1        2.95868        0.58590       5.05      0.0039

                         COMBINED LANGMUIR REGRESSION on TEMPERATURE=35
20
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           Obs            Temp               E               A      EbyA       Einv

            1              35           0.001           0.001      .          .
            2              35           0.493            0.45    1.09556    2.02840
            3              35           0.737            0.44    1.67500    1.35685
            4              35           0.976            0.83    1.17590    1.02459
            5              35           1.159            1.81    0.64033    0.86281
            6              35           1.412           2.516    0.56121    0.70822
            7              35           1.827           2.434    0.75062    0.54735
            8              35           2.472           2.989    0.82703    0.40453

           Obs      Ainv        LNE         LNA      CMBLNGE      PRED       RESID

            1      .           .           .          .          .           .
            2     2.22222    -0.70725    -0.79851    1.42422    2.41827    -0.19604
            3     2.27273    -0.30517    -0.82098    1.16484    1.65085     0.62188
            4     1.20482    -0.02429    -0.18633    1.01222    1.19930     0.00552
            5     0.55249     0.14756     0.59333    0.92888    0.95271    -0.40023
            6     0.39746     0.34501     0.92267    0.84156    0.69435    -0.29690
            7     0.41085     0.60268     0.88954    0.73983    0.39338     0.01747
            8     0.33456     0.90503     1.09494    0.63603    0.08626     0.24830

                            A versus E REGRESSION on TEMPERATURE=45
21
                                                                18:08 Saturday, October
25, 2003

                                       The REG Procedure
                                         Model: MODEL1
                                     Dependent Variable: A

                                      Analysis of Variance

                                             Sum of           Mean
         Source                   DF        Squares         Square    F Value    Pr > F

         Model                     1        6.31416        6.31416     164.69    <.0001
         Error                     6        0.23004        0.03834
         Corrected Total           7        6.54420


                      Root MSE              0.19581    R-Square     0.9648
                      Dependent Mean        1.24150    Adj R-Sq     0.9590
                      Coeff Var            15.77174


                                      Parameter Estimates

                                   Parameter       Standard
              Variable     DF       Estimate          Error    t Value    Pr > |t|

              Intercept     1       -0.14548        0.12835      -1.13      0.3003
              E             1        1.22241        0.09525      12.83      <.0001

                            A versus E REGRESSION on TEMPERATURE=45
22
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           Obs            Temp               E               A      EbyA       Einv

            1              45           0.001           0.001      .          .
            2              45           0.493            0.47    1.04894    2.02840
            3              45           0.737            0.57    1.29298    1.35685
            4              45           0.976            1.24    0.78710    1.02459
            5              45           1.159            1.11    1.04414    0.86281
            6              45           1.412             1.6    0.88250    0.70822
            7              45           1.827           1.841    0.99240    0.54735
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           8              45           2.472             3.1    0.79742    0.40453

          Obs      Ainv        LNE         LNA      CMBLNGE      PRED         RESID

           1        .           .           .          .        -0.14425     0.14525
           2     2.12766    -0.70725    -0.75502    1.42422      0.45717     0.01283
           3     1.75439    -0.30517    -0.56212    1.16484      0.75544    -0.18544
           4     0.80645    -0.02429     0.21511    1.01222      1.04760     0.19240
           5     0.90090     0.14756     0.10436    0.92888      1.27130    -0.16130
           6     0.62500     0.34501     0.47000    0.84156      1.58057     0.01943
           7     0.54318     0.60268     0.61031    0.73983      2.08787    -0.24687
           8     0.32258     0.90503     1.13140    0.63603      2.87632     0.22368

                         EbyA versus E REGRESSION on TEMPERATURE=45
23
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                                     The REG Procedure
                                       Model: MODEL1
                                  Dependent Variable: EbyA

                                    Analysis of Variance

                                             Sum of          Mean
        Source                   DF         Squares        Square    F Value    Pr > F

        Model                     1         0.05711       0.05711       2.20    0.1982
        Error                     5         0.12989       0.02598
        Corrected Total           6         0.18700


                     Root MSE              0.16118    R-Square     0.3054
                     Dependent Mean        0.97792    Adj R-Sq     0.1665
                     Coeff Var            16.48137


                                    Parameter Estimates

                                    Parameter       Standard
             Variable     DF         Estimate          Error    t Value    Pr > |t|

             Intercept     1          1.16455        0.13983       8.33      0.0004
             E             1         -0.14393        0.09707      -1.48      0.1982

                         EbyA versus E REGRESSION on TEMPERATURE=45
24
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          Obs           Temp              E               A      EbyA       Einv

           1              45           0.001           0.001      .          .
           2              45           0.493            0.47    1.04894    2.02840
           3              45           0.737            0.57    1.29298    1.35685
           4              45           0.976            1.24    0.78710    1.02459
           5              45           1.159            1.11    1.04414    0.86281
           6              45           1.412             1.6    0.88250    0.70822
           7              45           1.827           1.841    0.99240    0.54735
           8              45           2.472             3.1    0.79742    0.40453

          Obs      Ainv        LNE         LNA      CMBLNGE      PRED        RESID

           1        .           .           .          .        1.16440       .
           2     2.12766    -0.70725    -0.75502    1.42422     1.09359    -0.04465
           3     1.75439    -0.30517    -0.56212    1.16484     1.05847     0.23452
           4     0.80645    -0.02429     0.21511    1.01222     1.02407    -0.23697
           5     0.90090     0.14756     0.10436    0.92888     0.99773     0.04642
           6     0.62500     0.34501     0.47000    0.84156     0.96131    -0.07881
           7     0.54318     0.60268     0.61031    0.73983     0.90158     0.09082
           8     0.32258     0.90503     1.13140    0.63603     0.80874    -0.01132
```

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                         LANGMUIR REGRESSION on TEMPERATURE=45
25
                                                          18:08 Saturday, October
25, 2003

                                   The REG Procedure
                                     Model: MODEL1
                                Dependent Variable: Ainv

                                  Analysis of Variance

                                         Sum of           Mean
         Source                 DF      Squares         Square    F Value    Pr > F

         Model                   1      2.51971        2.51971      71.76    0.0004
         Error                   5      0.17557        0.03511
         Corrected Total         6      2.69528


                      Root MSE              0.18739    R-Square     0.9349
                      Dependent Mean        1.01145    Adj R-Sq     0.9218
                      Coeff Var            18.52666


                                  Parameter Estimates

                                     Parameter       Standard
              Variable     DF       Estimate          Error    t Value    Pr > |t|

              Intercept     1       -0.14490        0.15379      -0.94      0.3893
              Einv          1        1.16757        0.13783       8.47      0.0004

                         LANGMUIR REGRESSION on TEMPERATURE=45
26
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           Obs           Temp              E              A      EbyA       Einv

            1              45          0.001          0.001       .          .
            2              45          0.493           0.47   1.04894    2.02840
            3              45          0.737           0.57   1.29298    1.35685
            4              45          0.976           1.24   0.78710    1.02459
            5              45          1.159           1.11   1.04414    0.86281
            6              45          1.412            1.6   0.88250    0.70822
            7              45          1.827          1.841   0.99240    0.54735
            8              45          2.472            3.1   0.79742    0.40453

           Obs      Ainv         LNE          LNA      CMBLNGE       PRED       RESID

            1        .           .            .          .           .           .
            2     2.12766    -0.70725    -0.75502    1.42422    2.22340    -0.09574
            3     1.75439    -0.30517    -0.56212    1.16484    1.43932     0.31507
            4     0.80645    -0.02429     0.21511    1.01222    1.05138    -0.24493
            5     0.90090     0.14756     0.10436    0.92888    0.86249     0.03841
            6     0.62500     0.34501     0.47000    0.84156    0.68199    -0.05699
            7     0.54318     0.60268     0.61031    0.73983    0.49416     0.04902
            8     0.32258     0.90503     1.13140    0.63603    0.32742    -0.00484

                        FREUNDLICH REGRESSION on TEMPERATURE=45
27
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                                   The REG Procedure
                                     Model: MODEL1
                                 Dependent Variable: LNA

                                  Analysis of Variance
```

```
                                 Sum of           Mean
Source                   DF     Squares         Square    F Value    Pr > F

Model                     1     2.47736        2.47736      96.22    0.0002
Error                     5     0.12873        0.02575
Corrected Total           6     2.60609


             Root MSE              0.16046    R-Square     0.9506
             Dependent Mean        0.17343    Adj R-Sq     0.9407
             Coeff Var            92.51701


                            Parameter Estimates

                         Parameter       Standard
     Variable     DF      Estimate          Error    t Value    Pr > |t|

     Intercept     1       0.01124        0.06286       0.18      0.8651
     LNE           1       1.17829        0.12012       9.81      0.0002

                  FREUNDLICH REGRESSION on TEMPERATURE=45
28
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25, 2003

   Obs           Temp              E               A      EbyA       Einv

     1             45          0.001           0.001     .          .
     2             45          0.493            0.47    1.04894    2.02840
     3             45          0.737            0.57    1.29298    1.35685
     4             45          0.976            1.24    0.78710    1.02459
     5             45          1.159            1.11    1.04414    0.86281
     6             45          1.412             1.6    0.88250    0.70822
     7             45          1.827           1.841    0.99240    0.54735
     8             45          2.472             3.1    0.79742    0.40453

   Obs      Ainv        LNE         LNA      CMBLNGE      PRED        RESID

     1       .           .           .          .           .          .
     2    2.12766    -0.70725    -0.75502    1.42422    -0.82210     0.06708
     3    1.75439    -0.30517    -0.56212    1.16484    -0.34833    -0.21379
     4    0.80645    -0.02429     0.21511    1.01222    -0.01738     0.23249
     5    0.90090     0.14756     0.10436    0.92888     0.18511    -0.08075
     6    0.62500     0.34501     0.47000    0.84156     0.41776     0.05224
     7    0.54318     0.60268     0.61031    0.73983     0.72137    -0.11106
     8    0.32258     0.90503     1.13140    0.63603     1.07763     0.05378

               COMBINED LANGMUIR REGRESSION on TEMPERATURE=45
29
                                                         18:08 Saturday, October
25, 2003

                            The REG Procedure
                              Model: MODEL1
                         Dependent Variable: Ainv

                           Analysis of Variance

                                 Sum of           Mean
Source                   DF     Squares         Square    F Value    Pr > F

Model                     1     2.50052        2.50052      64.20    0.0005
Error                     5     0.19476        0.03895
Corrected Total           6     2.69528


             Root MSE              0.19736    R-Square     0.9277
             Dependent Mean        1.01145    Adj R-Sq     0.9133
             Coeff Var            19.51271
```

```
                                      Parameter Estimates

                                   Parameter       Standard
              Variable     DF       Estimate          Error    t Value    Pr > |t|

              Intercept     1       -1.31710        0.30005      -4.39      0.0071
              CMBLNGE       1        2.41566        0.30150       8.01      0.0005

                         COMBINED LANGMUIR REGRESSION on TEMPERATURE=45
30
                                                                18:08 Saturday, October
25, 2003

           Obs            Temp                E               A      EbyA       Einv

            1               45           0.001           0.001      .          .
            2               45           0.493            0.47    1.04894    2.02840
            3               45           0.737            0.57    1.29298    1.35685
            4               45           0.976            1.24    0.78710    1.02459
            5               45           1.159            1.11    1.04414    0.86281
            6               45           1.412             1.6    0.88250    0.70822
            7               45           1.827           1.841    0.99240    0.54735
            8               45           2.472             3.1    0.79742    0.40453

           Obs      Ainv        LNE         LNA      CMBLNGE      PRED       RESID

            1      .           .           .          .          .           .
            2     2.12766    -0.70725    -0.75502    1.42422    2.12333     0.00433
            3     1.75439    -0.30517    -0.56212    1.16484    1.49676     0.25762
            4     0.80645    -0.02429     0.21511    1.01222    1.12809    -0.32163
            5     0.90090     0.14756     0.10436    0.92888    0.92676    -0.02586
            6     0.62500     0.34501     0.47000    0.84156    0.71582    -0.09082
            7     0.54318     0.60268     0.61031    0.73983    0.47008     0.07311
            8     0.32258     0.90503     1.13140    0.63603    0.21933     0.10325

                            A versus E REGRESSION on TEMPERATURE=55
31
                                                                18:08 Saturday, October
25, 2003

                                       The REG Procedure
                                         Model: MODEL1
                                     Dependent Variable: A

                                      Analysis of Variance

                                             Sum of           Mean
         Source                   DF        Squares         Square    F Value    Pr > F

         Model                     1        9.45160        9.45160     713.29    <.0001
         Error                     6        0.07950        0.01325
         Corrected Total           7        9.53111


                      Root MSE              0.11511    R-Square     0.9917
                      Dependent Mean        1.76550    Adj R-Sq     0.9903
                      Coeff Var             6.52007


                                      Parameter Estimates

                                   Parameter       Standard
              Variable     DF       Estimate          Error    t Value    Pr > |t|

              Intercept     1        0.06857        0.07545       0.91      0.3985
              E             1        1.49559        0.05600      26.71      <.0001

                            A versus E REGRESSION on TEMPERATURE=55
32
                                                                18:08 Saturday, October
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25, 2003

           Obs            Temp              E              A      EbyA       Einv

             1              55          0.001          0.001       .          .
             2              55          0.493           0.75    0.65733    2.02840
             3              55          0.737           1.19    0.61933    1.35685
             4              55          0.976            1.7    0.57412    1.02459
             5              55          1.159           1.89    0.61323    0.86281
             6              55          1.412          2.173    0.64979    0.70822
             7              55          1.827           2.62    0.69733    0.54735
             8              55          2.472            3.8    0.65053    0.40453

           Obs      Ainv        LNE         LNA      CMBLNGE      PRED       RESID

             1       .           .           .          .        0.07007    -0.06907
             2     1.33333    -0.70725    -0.28768    1.42422    0.80589    -0.05589
             3     0.84034    -0.30517     0.17395    1.16484    1.17082     0.01918
             4     0.58824    -0.02429     0.53063    1.01222    1.52826     0.17174
             5     0.52910     0.14756     0.63658    0.92888    1.80195     0.08805
             6     0.46019     0.34501     0.77611    0.84156    2.18034    -0.00734
             7     0.38168     0.60268     0.96317    0.73983    2.80101    -0.18101
             8     0.26316     0.90503     1.33500    0.63603    3.76566     0.03434

                          EbyA versus E REGRESSION on TEMPERATURE=55
33
                                                                     18:08 Saturday, October
25, 2003

                                     The REG Procedure
                                       Model: MODEL1
                                 Dependent Variable: EbyA

                                    Analysis of Variance

                                             Sum of           Mean
         Source                   DF        Squares         Square    F Value    Pr > F

         Model                     1        0.00160        0.00160       1.05    0.3533
         Error                     5        0.00763        0.00153
         Corrected Total           6        0.00923


                      Root MSE              0.03907    R-Square     0.1730
                      Dependent Mean        0.63738    Adj R-Sq     0.0076
                      Coeff Var             6.13011


                                    Parameter Estimates

                                     Parameter       Standard
              Variable     DF         Estimate          Error    t Value    Pr > |t|

              Intercept     1          0.60617        0.03390      17.88      <.0001
              E             1          0.02407        0.02353       1.02      0.3533

                          EbyA versus E REGRESSION on TEMPERATURE=55
34
                                                                     18:08 Saturday, October
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           Obs            Temp              E              A      EbyA       Einv

             1              55          0.001          0.001       .          .
             2              55          0.493           0.75    0.65733    2.02840
             3              55          0.737           1.19    0.61933    1.35685
             4              55          0.976            1.7    0.57412    1.02459
             5              55          1.159           1.89    0.61323    0.86281
             6              55          1.412          2.173    0.64979    0.70822
             7              55          1.827           2.62    0.69733    0.54735
             8              55          2.472            3.8    0.65053    0.40453
```

```
           Obs      Ainv        LNE         LNA      CMBLNGE      PRED       RESID

            1      .           .           .          .         0.60620      .
            2     1.33333   -0.70725   -0.28768    1.42422    0.61804     0.039295
            3     0.84034   -0.30517    0.17395    1.16484    0.62391    -0.004583
            4     0.58824   -0.02429    0.53063    1.01222    0.62966    -0.055546
            5     0.52910    0.14756    0.63658    0.92888    0.63407    -0.020840
            6     0.46019    0.34501    0.77611    0.84156    0.64016     0.009636
            7     0.38168    0.60268    0.96317    0.73983    0.65015     0.047182
            8     0.26316    0.90503    1.33500    0.63603    0.66567    -0.015144

                             LANGMUIR REGRESSION on TEMPERATURE=55
35
                                                                18:08 Saturday, October
25, 2003

                                       The REG Procedure
                                         Model: MODEL1
                                   Dependent Variable: Ainv

                                      Analysis of Variance

                                             Sum of           Mean
         Source                   DF        Squares         Square    F Value    Pr > F

         Model                     1        0.76800        0.76800     486.96    <.0001
         Error                     5        0.00789        0.00158
         Corrected Total           6        0.77589


                      Root MSE              0.03971    R-Square     0.9898
                      Dependent Mean        0.62801    Adj R-Sq     0.9878
                      Coeff Var             6.32367


                                      Parameter Estimates

                                   Parameter       Standard
              Variable     DF       Estimate          Error    t Value    Pr > |t|

              Intercept     1       -0.01040        0.03259      -0.32      0.7625
              Einv          1        0.64460        0.02921      22.07      <.0001

                             LANGMUIR REGRESSION on TEMPERATURE=55
36
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25, 2003

           Obs            Temp               E               A      EbyA       Einv

            1               55           0.001           0.001     .          .
            2               55           0.493            0.75    0.65733    2.02840
            3               55           0.737            1.19    0.61933    1.35685
            4               55           0.976             1.7    0.57412    1.02459
            5               55           1.159            1.89    0.61323    0.86281
            6               55           1.412           2.173    0.64979    0.70822
            7               55           1.827            2.62    0.69733    0.54735
            8               55           2.472             3.8    0.65053    0.40453

           Obs      Ainv        LNE         LNA      CMBLNGE      PRED       RESID

            1      .           .           .          .          .           .
            2     1.33333   -0.70725   -0.28768    1.42422    1.29710     0.036231
            3     0.84034   -0.30517    0.17395    1.16484    0.86423    -0.023889
            4     0.58824   -0.02429    0.53063    1.01222    0.65005    -0.061814
            5     0.52910    0.14756    0.63658    0.92888    0.54577    -0.016667
            6     0.46019    0.34501    0.77611    0.84156    0.44611     0.014079
            7     0.38168    0.60268    0.96317    0.73983    0.34242     0.039262
            8     0.26316    0.90503    1.33500    0.63603    0.25036     0.012798
```

```
                    FREUNDLICH REGRESSION on TEMPERATURE=55
37
                                                    18:08 Saturday, October
25, 2003

                               The REG Procedure
                                 Model: MODEL1
                            Dependent Variable: LNA

                              Analysis of Variance

                                      Sum of           Mean
         Source                DF        Squares         Square    F Value    Pr > F

         Model                  1        1.65734        1.65734     400.33    <.0001
         Error                  5        0.02070        0.00414
         Corrected Total        6        1.67804


                      Root MSE              0.06434    R-Square     0.9877
                      Dependent Mean        0.58968    Adj R-Sq     0.9852
                      Coeff Var            10.91142


                              Parameter Estimates

                                Parameter       Standard
              Variable     DF      Estimate          Error    t Value    Pr > |t|

              Intercept     1       0.45702        0.02521      18.13      <.0001
              LNE           1       0.96375        0.04817      20.01      <.0001

                    FREUNDLICH REGRESSION on TEMPERATURE=55
38
                                                    18:08 Saturday, October
25, 2003

           Obs           Temp              E              A      EbyA       Einv

            1             55           0.001          0.001       .          .
            2             55           0.493           0.75    0.65733    2.02840
            3             55           0.737           1.19    0.61933    1.35685
            4             55           0.976            1.7    0.57412    1.02459
            5             55           1.159           1.89    0.61323    0.86281
            6             55           1.412          2.173    0.64979    0.70822
            7             55           1.827           2.62    0.69733    0.54735
            8             55           2.472            3.8    0.65053    0.40453

           Obs      Ainv         LNE          LNA      CMBLNGE       PRED         RESID

            1         .            .            .          .           .            .
            2     1.33333    -0.70725    -0.28768    1.42422    -0.22459    -0.063096
            3     0.84034    -0.30517     0.17395    1.16484     0.16292     0.011038
            4     0.58824    -0.02429     0.53063    1.01222     0.43361     0.097021
            5     0.52910     0.14756     0.63658    0.92888     0.59923     0.037350
            6     0.46019     0.34501     0.77611    0.84156     0.78952    -0.013409
            7     0.38168     0.60268     0.96317    0.73983     1.03784    -0.074670
            8     0.26316     0.90503     1.33500    0.63603     1.32924     0.005766

                 COMBINED LANGMUIR REGRESSION on TEMPERATURE=55
39
                                                    18:08 Saturday, October
25, 2003

                               The REG Procedure
                                 Model: MODEL1
                           Dependent Variable: Ainv

                              Analysis of Variance

                                      Sum of           Mean
```

```
Source                  DF        Squares         Square    F Value    Pr > F

Model                    1        0.74295        0.74295     112.77    0.0001
Error                    5        0.03294        0.00659
Corrected Total          6        0.77589


             Root MSE              0.08117    R-Square     0.9575
             Dependent Mean        0.62801    Adj R-Sq     0.9491
             Coeff Var            12.92472


                             Parameter Estimates

                              Parameter       Standard
     Variable      DF          Estimate          Error    t Value    Pr > |t|

     Intercept      1          -0.64125        0.12340      -5.20      0.0035
     CMBLNGE        1           1.31674        0.12400      10.62      0.0001
                     COMBINED LANGMUIR REGRESSION on TEMPERATURE=55
40
                                                        18:08 Saturday, October
25, 2003

       Obs            Temp              E               A      EbyA       Einv

        1              55           0.001           0.001       .          .
        2              55           0.493            0.75    0.65733    2.02840
        3              55           0.737            1.19    0.61933    1.35685
        4              55           0.976             1.7    0.57412    1.02459
        5              55           1.159            1.89    0.61323    0.86281
        6              55           1.412           2.173    0.64979    0.70822
        7              55           1.827            2.62    0.69733    0.54735
        8              55           2.472             3.8    0.65053    0.40453

       Obs      Ainv         LNE          LNA      CMBLNGE       PRED       RESID

        1         .           .            .          .           .           .
        2      1.33333    -0.70725    -0.28768    1.42422    1.23407     0.09926
        3      0.84034    -0.30517     0.17395    1.16484    0.89254    -0.05220
        4      0.58824    -0.02429     0.53063    1.01222    0.69158    -0.10335
        5      0.52910     0.14756     0.63658    0.92888    0.58184    -0.05274
        6      0.46019     0.34501     0.77611    0.84156    0.46686    -0.00667
        7      0.38168     0.60268     0.96317    0.73983    0.33291     0.04877
        8      0.26316     0.90503     1.33500    0.63603    0.19623     0.06692
```

Appendix B

Statistical Analysis for Experimental Adsorption Data of CBH II to fit Adsorption Models

SAS CODE

```
%let path=D:\Dole Files\;
libname bus "&path.";
/* THIS SAS FILE IS FOR REGRESSION FOR DATA ON CBHIIa FILE */

/* This step makes new variables for calculating different models */

data temp;
set bus.CBHIIa;
if E>0.001 then do;
m=2;
EbyA=E/A;
Einv=1/E;
Ainv=1/A;
LNE=log(E);
LNA=log(A);
CMBLNGE=1/(E**(1/m));
drop m;
end;
run;


/*********************************************/
/* regression models ..... FOR TEMPERATURE=25 */
/*********************************************/

/* Selecting Cases */

data temp25;
set temp;
if TEMP=25 then output;
run;

/* A versus E Regression */
title 'A versus E REGRESSION on TEMPERATURE=25';
PROC REG data=temp25;
  MODEL A=E;
OUTPUT OUT=AE P=PRED R=RESID;
PLOT A*E='*' PREDICTED.*E='o' RESIDUAL.*E='x';
PROC PRINT DATA=AE;
run;


/* EbyA versus E Regression */
title 'EbyA versus E REGRESSION on TEMPERATURE=25';
PROC REG data=temp25;
  MODEL EbyA=E;
OUTPUT OUT=EbyA P=PRED R=RESID;
PLOT EbyA*E='*' PREDICTED.*E='o' RESIDUAL.*E='x';
PROC PRINT DATA=EbyA;
run;


/* Langmuir Regression */
title 'LANGMUIR REGRESSION on TEMPERATURE=25';
PROC REG data=temp25;
```

```
  MODEL Ainv=Einv;
OUTPUT OUT=LANGMUIR P=PRED R=RESID;
PLOT Ainv*Einv='*' PREDICTED.*Einv='o' RESIDUAL.*Einv='x';
PROC PRINT DATA=LANGMUIR;
run;

/* Freundlich Regression */
title 'FREUNDLICH REGRESSION on TEMPERATURE=25';
PROC REG data=temp25;
MODEL LNA=LNE;
OUTPUT OUT=FREUNDLICH P=PRED R=RESID;
PLOT LNA*LNE='*' PREDICTED.*LNE='o' RESIDUAL.*LNE='x';
PROC PRINT DATA=FREUNDLICH;
run;

/* Combined Langmuir Regression */
title 'COMBINED LANGMUIR REGRESSION on TEMPERATURE=25';
PROC REG data=temp25;
MODEL Ainv=CMBLNGE;
OUTPUT OUT=COMBLANGMUIR P=PRED R=RESID;
PLOT Ainv*CMBLNGE='*' PREDICTED.*CMBLNGE='o' RESIDUAL.*CMBLNGE='x';
PROC PRINT DATA=COMBLANGMUIR;
run;


/**********************************************/
/* regression models ..... FOR TEMPERATURE=35 */
/**********************************************/

/* Selecting Cases */

data temp35;
set temp;
if TEMP=35 then output;
run;

/* A versus E Regression */
title 'A versus E REGRESSION on TEMPERATURE=35';
PROC REG data=temp35;
  MODEL A=E;
OUTPUT OUT=AE P=PRED R=RESID;
PLOT A*E='*' PREDICTED.*E='o' RESIDUAL.*E='x';
PROC PRINT DATA=AE;
run;

/* EbyA versus E Regression */
title 'EbyA versus E REGRESSION on TEMPERATURE=35';
PROC REG data=temp35;
  MODEL EbyA=E;
OUTPUT OUT=EbyA P=PRED R=RESID;
PLOT EbyA*E='*' PREDICTED.*E='o' RESIDUAL.*E='x';
PROC PRINT DATA=EbyA;
run;

/* Langmuir Regression */
title 'LANGMUIR REGRESSION on TEMPERATURE=35';
PROC REG data=temp35;
```

```
  MODEL Ainv=Einv;
OUTPUT OUT=LANGMUIR P=PRED R=RESID;
PLOT Ainv*Einv='*' PREDICTED.*Einv='o' RESIDUAL.*Einv='x';
PROC PRINT DATA=LANGMUIR;
run;

/* Freundlich Regression */
title 'FREUNDLICH REGRESSION on TEMPERATURE=35';
PROC REG data=temp35;
MODEL LNA=LNE;
OUTPUT OUT=FREUNDLICH P=PRED R=RESID;
PLOT LNA*LNE='*' PREDICTED.*LNE='o' RESIDUAL.*LNE='x';
PROC PRINT DATA=FREUNDLICH;
run;

/* Combined Langmuir Regression */
title 'COMBINED LANGMUIR REGRESSION on TEMPERATURE=35';
PROC REG data=temp35;
MODEL Ainv=CMBLNGE;
OUTPUT OUT=COMBLANGMUIR P=PRED R=RESID;
PLOT Ainv*CMBLNGE='*' PREDICTED.*CMBLNGE='o' RESIDUAL.*CMBLNGE='x';
PROC PRINT DATA=COMBLANGMUIR;
run;


/*********************************************/
/* regression models ..... FOR TEMPERATURE=45 */
/*********************************************/

/* Selecting Cases */

data temp45;
set temp;
if TEMP=45 then output;
run;

/* A versus E Regression */
title 'A versus E REGRESSION on TEMPERATURE=45';
PROC REG data=temp45;
  MODEL A=E;
OUTPUT OUT=AE P=PRED R=RESID;
PLOT A*E='*' PREDICTED.*E='o' RESIDUAL.*E='x';
PROC PRINT DATA=AE;
run;


/* EbyA versus E Regression */
title 'EbyA versus E REGRESSION on TEMPERATURE=45';
PROC REG data=temp45;
  MODEL EbyA=E;
OUTPUT OUT=EbyA P=PRED R=RESID;
PLOT EbyA*E='*' PREDICTED.*E='o' RESIDUAL.*E='x';
PROC PRINT DATA=EbyA;
run;

/* Langmuir Regression */
title 'LANGMUIR REGRESSION on TEMPERATURE=45';
```

```
PROC REG data=temp45;
  MODEL Ainv=Einv;
OUTPUT OUT=LANGMUIR P=PRED R=RESID;
PLOT Ainv*Einv='*' PREDICTED.*Einv='o' RESIDUAL.*Einv='x';
PROC PRINT DATA=LANGMUIR;
run;

/* Freundlich Regression */
title 'FREUNDLICH REGRESSION on TEMPERATURE=45';
PROC REG data=temp45;
MODEL LNA=LNE;
OUTPUT OUT=FREUNDLICH P=PRED R=RESID;
PLOT LNA*LNE='*' PREDICTED.*LNE='o' RESIDUAL.*LNE='x';
PROC PRINT DATA=FREUNDLICH;
run;

/* Combined Langmuir Regression */
title 'COMBINED LANGMUIR REGRESSION on TEMPERATURE=45';
PROC REG data=temp45;
MODEL Ainv=CMBLNGE;
OUTPUT OUT=COMBLANGMUIR P=PRED R=RESID;
PLOT Ainv*CMBLNGE='*' PREDICTED.*CMBLNGE='o' RESIDUAL.*CMBLNGE='x';
PROC PRINT DATA=COMBLANGMUIR;
run;


/**********************************************/
/* regression models ..... FOR TEMPERATURE=55 */
/**********************************************/

/* Selecting Cases */

data temp55;
set temp;
if TEMP=55 then output;
run;

/* A versus E Regression */
title 'A versus E REGRESSION on TEMPERATURE=55';
PROC REG data=temp55;
  MODEL A=E;
OUTPUT OUT=AE P=PRED R=RESID;
PLOT A*E='*' PREDICTED.*E='o' RESIDUAL.*E='x';
PROC PRINT DATA=AE;
run;

/* EbyA versus E Regression */
title 'EbyA versus E REGRESSION on TEMPERATURE=55';
PROC REG data=temp55;
  MODEL EbyA=E;
OUTPUT OUT=EbyA P=PRED R=RESID;
PLOT EbyA*E='*' PREDICTED.*E='o' RESIDUAL.*E='x';
PROC PRINT DATA=EbyA;
run;

/* Langmuir Regression */
title 'LANGMUIR REGRESSION on TEMPERATURE=55';
```

```
PROC REG data=temp55;
  MODEL Ainv=Einv;
OUTPUT OUT=LANGMUIR P=PRED R=RESID;
PLOT Ainv*Einv='*' PREDICTED.*Einv='o' RESIDUAL.*Einv='x';
PROC PRINT DATA=LANGMUIR;
run;

/* Freundlich Regression */
title 'FREUNDLICH REGRESSION on TEMPERATURE=55';
PROC REG data=temp55;
MODEL LNA=LNE;
OUTPUT OUT=FREUNDLICH P=PRED R=RESID;
PLOT LNA*LNE='*' PREDICTED.*LNE='o' RESIDUAL.*LNE='x';
PROC PRINT DATA=FREUNDLICH;
run;

/* Combined Langmuir Regression */
title 'COMBINED LANGMUIR REGRESSION on TEMPERATURE=55';
PROC REG data=temp55;
MODEL Ainv=CMBLNGE;
OUTPUT OUT=COMBLANGMUIR P=PRED R=RESID;
PLOT Ainv*CMBLNGE='*' PREDICTED.*CMBLNGE='o' RESIDUAL.*CMBLNGE='x';
PROC PRINT DATA=COMBLANGMUIR;
run;
```

SAS OUTPUT

```
                            A versus E REGRESSION on TEMPERATURE=25
1
                                                                18:32 Saturday, October
25, 2003

                                       The REG Procedure
                                         Model: MODEL1
                                     Dependent Variable: A

                                      Analysis of Variance

                                             Sum of           Mean
         Source                   DF        Squares         Square    F Value    Pr > F

         Model                     1       13.75884       13.75884      70.23    0.0002
         Error                     6        1.17549        0.19592
         Corrected Total           7       14.93433


                      Root MSE              0.44262    R-Square     0.9213
                      Dependent Mean        1.80025    Adj R-Sq     0.9082
                      Coeff Var            24.58677


                                      Parameter Estimates

                                   Parameter       Standard
              Variable     DF       Estimate          Error    t Value    Pr > |t|

              Intercept     1       -0.60548        0.32695      -1.85      0.1135
              E             1        2.40302        0.28675       8.38      0.0002

                            A versus E REGRESSION on TEMPERATURE=25
2
                                                                18:32 Saturday, October
25, 2003

           Obs            Temp               E               A      EbyA       Einv

            1              25           0.001           0.001     .          .
            2              25           0.477             0.3    1.59000    2.09644
            3              25           0.715            0.52    1.37500    1.39860
            4              25           0.929            1.44    0.64514    1.07643
            5              25           1.315            2.19    0.60046    0.76046
            6              25           1.335           2.763    0.48317    0.74906
            7              25           1.463           3.308    0.44226    0.68353
            8              25           1.774            3.88    0.45722    0.56370

           Obs      Ainv        LNE         LNA      CMBLNGE      PRED        RESID

            1      .           .           .          .         -0.60307     0.60407
            2     3.33333    -0.74024    -1.20397    1.44791     0.54077    -0.24077
            3     1.92308    -0.33547    -0.65393    1.18262     1.11268    -0.59268
            4     0.69444    -0.07365     0.36464    1.03751     1.62693    -0.18693
            5     0.45662     0.27384     0.78390    0.87204     2.55450    -0.36450
            6     0.36193     0.28893     1.01632    0.86548     2.60256     0.16044
            7     0.30230     0.38049     1.19634    0.82676     2.91015     0.39785
            8     0.25773     0.57324     1.35584    0.75080     3.65749     0.22251

                           EbyA versus E REGRESSION on TEMPERATURE=25
3
                                                                18:32 Saturday, October
25, 2003

                                       The REG Procedure
                                         Model: MODEL1
                                   Dependent Variable: EbyA
```

```
                                      Analysis of Variance

                                             Sum of           Mean
         Source                   DF        Squares         Square    F Value    Pr > F

         Model                     1        1.09498        1.09498      20.32    0.0064
         Error                     5        0.26940        0.05388
         Corrected Total           6        1.36438


                      Root MSE              0.23212    R-Square     0.8025
                      Dependent Mean        0.79903    Adj R-Sq     0.7631
                      Coeff Var            29.05000


                                      Parameter Estimates

                                   Parameter       Standard
              Variable     DF       Estimate          Error    t Value    Pr > |t|

              Intercept     1        1.87426        0.25413       7.38      0.0007
              E             1       -0.93988        0.20849      -4.51      0.0064

                           EbyA versus E REGRESSION on TEMPERATURE=25
4
                                                                18:32 Saturday, October
25, 2003

           Obs            Temp               E               A      EbyA       Einv

            1              25           0.001           0.001     .          .
            2              25           0.477             0.3    1.59000    2.09644
            3              25           0.715            0.52    1.37500    1.39860
            4              25           0.929            1.44    0.64514    1.07643
            5              25           1.315            2.19    0.60046    0.76046
            6              25           1.335           2.763    0.48317    0.74906
            7              25           1.463           3.308    0.44226    0.68353
            8              25           1.774            3.88    0.45722    0.56370

           Obs      Ainv        LNE          LNA      CMBLNGE      PRED       RESID

            1      .          .            .          .         1.87332      .
            2     3.33333    -0.74024    -1.20397    1.44791    1.42594     0.16406
            3     1.92308    -0.33547    -0.65393    1.18262    1.20224     0.17276
            4     0.69444    -0.07365     0.36464    1.03751    1.00111    -0.35597
            5     0.45662     0.27384     0.78390    0.87204    0.63832    -0.03786
            6     0.36193     0.28893     1.01632    0.86548    0.61952    -0.13635
            7     0.30230     0.38049     1.19634    0.82676    0.49921    -0.05695
            8     0.25773     0.57324     1.35584    0.75080    0.20691     0.25031

                             LANGMUIR REGRESSION on TEMPERATURE=25
5
                                                                18:32 Saturday, October
25, 2003

                                       The REG Procedure
                                         Model: MODEL1
                                   Dependent Variable: Ainv

                                      Analysis of Variance

                                             Sum of           Mean
         Source                   DF        Squares         Square    F Value    Pr > F

         Model                     1        7.86240        7.86240     155.92    <.0001
         Error                     5        0.25213        0.05043
         Corrected Total           6        8.11453


                      Root MSE              0.22456    R-Square     0.9689
                      Dependent Mean        1.04706    Adj R-Sq     0.9627
```

```
                      Coeff Var           21.44637


                                      Parameter Estimates

                                   Parameter       Standard
              Variable     DF       Estimate          Error    t Value    Pr > |t|

              Intercept     1       -1.16414        0.19637      -5.93      0.0019
              Einv          1        2.11217        0.16915      12.49      <.0001

                                LANGMUIR REGRESSION on TEMPERATURE=25
6
                                                                  18:32 Saturday, October
25, 2003

           Obs            Temp               E               A      EbyA       Einv

             1              25           0.001           0.001     .          .
             2              25           0.477             0.3    1.59000    2.09644
             3              25           0.715            0.52    1.37500    1.39860
             4              25           0.929            1.44    0.64514    1.07643
             5              25           1.315            2.19    0.60046    0.76046
             6              25           1.335           2.763    0.48317    0.74906
             7              25           1.463           3.308    0.44226    0.68353
             8              25           1.774            3.88    0.45722    0.56370

           Obs      Ainv        LNE          LNA      CMBLNGE      PRED       RESID

             1      .           .            .          .          .           .
             2    3.33333    -0.74024    -1.20397    1.44791    3.26389     0.06945
             3    1.92308    -0.33547    -0.65393    1.18262    1.78994     0.13313
             4    0.69444    -0.07365     0.36464    1.03751    1.10945    -0.41501
             5    0.45662     0.27384     0.78390    0.87204    0.44207     0.01455
             6    0.36193     0.28893     1.01632    0.86548    0.41801    -0.05608
             7    0.30230     0.38049     1.19634    0.82676    0.27958     0.02271
             8    0.25773     0.57324     1.35584    0.75080    0.02648     0.23125

                               FREUNDLICH REGRESSION on TEMPERATURE=25
7
                                                                  18:32 Saturday, October
25, 2003

                                       The REG Procedure
                                         Model: MODEL1
                                    Dependent Variable: LNA

                                      Analysis of Variance

                                            Sum of           Mean
         Source                    DF        Squares         Square    F Value    Pr > F

         Model                      1        5.59482        5.59482     170.12    <.0001
         Error                      5        0.16444        0.03289
         Corrected Total            6        5.75925


                      Root MSE              0.18135    R-Square     0.9714
                      Dependent Mean        0.40845    Adj R-Sq     0.9657
                      Coeff Var            44.39924


                                      Parameter Estimates

                                   Parameter       Standard
              Variable     DF       Estimate          Error    t Value    Pr > |t|

              Intercept     1        0.29873        0.06906       4.33      0.0075
              LNE           1        2.09190        0.16038      13.04      <.0001

                               FREUNDLICH REGRESSION on TEMPERATURE=25
```

```
8
                                                         18:32 Saturday, October
25, 2003

          Obs          Temp             E              A      EbyA       Einv

            1            25         0.001          0.001       .          .
            2            25         0.477            0.3    1.59000    2.09644
            3            25         0.715           0.52    1.37500    1.39860
            4            25         0.929           1.44    0.64514    1.07643
            5            25         1.315           2.19    0.60046    0.76046
            6            25         1.335          2.763    0.48317    0.74906
            7            25         1.463          3.308    0.44226    0.68353
            8            25         1.774           3.88    0.45722    0.56370

          Obs      Ainv        LNE         LNA      CMBLNGE      PRED        RESID

            1      .          .           .          .          .            .
            2    3.33333   -0.74024    -1.20397    1.44791    -1.24978     0.04580
            3    1.92308   -0.33547    -0.65393    1.18262    -0.40304    -0.25088
            4    0.69444   -0.07365     0.36464    1.03751     0.14467     0.21997
            5    0.45662    0.27384     0.78390    0.87204     0.87157    -0.08767
            6    0.36193    0.28893     1.01632    0.86548     0.90315     0.11317
            7    0.30230    0.38049     1.19634    0.82676     1.09468     0.10166
            8    0.25773    0.57324     1.35584    0.75080     1.49789    -0.14205

                        COMBINED LANGMUIR REGRESSION on TEMPERATURE=25
9
                                                         18:32 Saturday, October
25, 2003

                                      The REG Procedure
                                        Model: MODEL1
                                   Dependent Variable: Ainv

                                     Analysis of Variance

                                            Sum of           Mean
        Source                   DF        Squares         Square    F Value    Pr > F

        Model                     1        7.65270        7.65270      82.85    0.0003
        Error                     5        0.46183        0.09237
        Corrected Total           6        8.11453


                     Root MSE              0.30392    R-Square     0.9431
                     Dependent Mean        1.04706    Adj R-Sq     0.9317
                     Coeff Var            29.02578


                                     Parameter Estimates

                                 Parameter       Standard
             Variable     DF      Estimate          Error    t Value    Pr > |t|

             Intercept     1      -3.54018        0.51689      -6.85      0.0010
             CMBLNGE       1       4.59833        0.50518       9.10      0.0003

                        COMBINED LANGMUIR REGRESSION on TEMPERATURE=25
10
                                                         18:32 Saturday, October
25, 2003

          Obs          Temp             E              A      EbyA       Einv

            1            25         0.001          0.001       .          .
            2            25         0.477            0.3    1.59000    2.09644
            3            25         0.715           0.52    1.37500    1.39860
            4            25         0.929           1.44    0.64514    1.07643
            5            25         1.315           2.19    0.60046    0.76046
            6            25         1.335          2.763    0.48317    0.74906
```

```
            7               25           1.463           3.308    0.44226    0.68353
            8               25           1.774            3.88    0.45722    0.56370

           Obs      Ainv        LNE         LNA      CMBLNGE      PRED        RESID

            1      .           .           .          .           .           .
            2     3.33333    -0.74024    -1.20397     1.44791     3.11777     0.21556
            3     1.92308    -0.33547    -0.65393     1.18262     1.89792     0.02516
            4     0.69444    -0.07365     0.36464     1.03751     1.23063    -0.53619
            5     0.45662     0.27384     0.78390     0.87204     0.46975    -0.01313
            6     0.36193     0.28893     1.01632     0.86548     0.43960    -0.07768
            7     0.30230     0.38049     1.19634     0.82676     0.26152     0.04078
            8     0.25773     0.57324     1.35584     0.75080    -0.08776     0.34550

                            A versus E REGRESSION on TEMPERATURE=35
11
                                                                18:32 Saturday, October
25, 2003

                                       The REG Procedure
                                         Model: MODEL1
                                     Dependent Variable: A

                                      Analysis of Variance

                                             Sum of           Mean
         Source                   DF        Squares         Square    F Value    Pr > F

         Model                     1       16.70985       16.70985      80.05    0.0001
         Error                     6        1.25238        0.20873
         Corrected Total           7       17.96223


                      Root MSE              0.45687    R-Square     0.9303
                      Dependent Mean        2.01625    Adj R-Sq     0.9187
                      Coeff Var            22.65942


                                      Parameter Estimates

                                   Parameter       Standard
              Variable     DF       Estimate          Error    t Value    Pr > |t|

              Intercept     1       -0.63494        0.33748      -1.88      0.1089
              E             1        2.64822        0.29598       8.95      0.0001

                            A versus E REGRESSION on TEMPERATURE=35
12
                                                                18:32 Saturday, October
25, 2003

           Obs            Temp               E               A      EbyA       Einv

            1               35           0.001           0.001     .          .
            2               35           0.477            0.51    0.93529    2.09644
            3               35           0.715             0.6    1.19167    1.39860
            4               35           0.929            1.56    0.59551    1.07643
            5               35           1.315            2.66    0.49436    0.76046
            6               35           1.335            3.03    0.44059    0.74906
            7               35           1.463           3.179    0.46021    0.68353
            8               35           1.774            4.59    0.38649    0.56370

           Obs      Ainv        LNE         LNA      CMBLNGE      PRED        RESID

            1      .           .           .          .         -0.63230     0.63330
            2     1.96078    -0.74024    -0.67334     1.44791     0.62825    -0.11825
            3     1.66667    -0.33547    -0.51083     1.18262     1.25853    -0.65853
            4     0.64103    -0.07365     0.44469     1.03751     1.82525    -0.26525
            5     0.37594     0.27384     0.97833     0.87204     2.84746    -0.18746
            6     0.33003     0.28893     1.10856     0.86548     2.90042     0.12958
            7     0.31456     0.38049     1.15657     0.82676     3.23939    -0.06039
```

```
          8     0.21786     0.57324     1.52388    0.75080     4.06299     0.52701

                           EbyA versus E REGRESSION on TEMPERATURE=35
13
                                                                   18:32 Saturday, October
25, 2003

                                       The REG Procedure
                                         Model: MODEL1
                                   Dependent Variable: EbyA

                                      Analysis of Variance

                                             Sum of           Mean
         Source                   DF        Squares         Square    F Value    Pr > F

         Model                     1        0.39997        0.39997      13.24    0.0149
         Error                     5        0.15102        0.03020
         Corrected Total           6        0.55100


                      Root MSE              0.17380    R-Square     0.7259
                      Dependent Mean        0.64345    Adj R-Sq     0.6711
                      Coeff Var            27.01010


                                      Parameter Estimates

                                   Parameter       Standard
              Variable     DF       Estimate          Error    t Value    Pr > |t|

              Intercept     1        1.29329        0.19028       6.80      0.0010
              E             1       -0.56805        0.15610      -3.64      0.0149

                           EbyA versus E REGRESSION on TEMPERATURE=35
14
                                                                   18:32 Saturday, October
25, 2003

          Obs           Temp              E              A      EbyA       Einv

            1             35          0.001          0.001     .          .
            2             35          0.477           0.51    0.93529    2.09644
            3             35          0.715            0.6    1.19167    1.39860
            4             35          0.929           1.56    0.59551    1.07643
            5             35          1.315           2.66    0.49436    0.76046
            6             35          1.335           3.03    0.44059    0.74906
            7             35          1.463          3.179    0.46021    0.68353
            8             35          1.774           4.59    0.38649    0.56370

          Obs      Ainv        LNE         LNA      CMBLNGE      PRED       RESID

            1      .           .           .          .        1.29272      .
            2     1.96078    -0.74024    -0.67334    1.44791    1.02233    -0.08704
            3     1.66667    -0.33547    -0.51083    1.18262    0.88714     0.30453
            4     0.64103    -0.07365     0.44469    1.03751    0.76558    -0.17006
            5     0.37594     0.27384     0.97833    0.87204    0.54631    -0.05195
            6     0.33003     0.28893     1.10856    0.86548    0.53495    -0.09436
            7     0.31456     0.38049     1.15657    0.82676    0.46224    -0.00203
            8     0.21786     0.57324     1.52388    0.75080    0.28558     0.10092

                             LANGMUIR REGRESSION on TEMPERATURE=35
15
                                                                   18:32 Saturday, October
25, 2003

                                       The REG Procedure
                                         Model: MODEL1
                                   Dependent Variable: Ainv

                                      Analysis of Variance
```

```
                                             Sum of           Mean
         Source                   DF        Squares         Square    F Value    Pr > F

         Model                     1        2.84078        2.84078      55.27    0.0007
         Error                     5        0.25701        0.05140
         Corrected Total           6        3.09779


                      Root MSE              0.22672    R-Square     0.9170
                      Dependent Mean        0.78670    Adj R-Sq     0.9004
                      Coeff Var            28.81929


                                      Parameter Estimates

                                   Parameter       Standard
              Variable     DF       Estimate          Error    t Value    Pr > |t|

              Intercept     1       -0.54244        0.19826      -2.74      0.0410
              Einv          1        1.26961        0.17078       7.43      0.0007

                             LANGMUIR REGRESSION on TEMPERATURE=35
16
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25, 2003

           Obs           Temp              E               A      EbyA       Einv

            1             35           0.001           0.001     .          .
            2             35           0.477            0.51    0.93529    2.09644
            3             35           0.715             0.6    1.19167    1.39860
            4             35           0.929            1.56    0.59551    1.07643
            5             35           1.315            2.66    0.49436    0.76046
            6             35           1.335            3.03    0.44059    0.74906
            7             35           1.463           3.179    0.46021    0.68353
            8             35           1.774            4.59    0.38649    0.56370

           Obs      Ainv        LNE         LNA      CMBLNGE      PRED       RESID

            1      .           .           .          .          .           .
            2    1.96078    -0.74024    -0.67334    1.44791    2.11921    -0.15843
            3    1.66667    -0.33547    -0.51083    1.18262    1.23324     0.43343
            4    0.64103    -0.07365     0.44469    1.03751    0.82420    -0.18317
            5    0.37594     0.27384     0.97833    0.87204    0.42304    -0.04710
            6    0.33003     0.28893     1.10856    0.86548    0.40858    -0.07854
            7    0.31456     0.38049     1.15657    0.82676    0.32537    -0.01081
            8    0.21786     0.57324     1.52388    0.75080    0.17324     0.04463

                            FREUNDLICH REGRESSION on TEMPERATURE=35
17
                                                                18:32 Saturday, October
25, 2003

                                       The REG Procedure
                                         Model: MODEL1
                                    Dependent Variable: LNA

                                      Analysis of Variance

                                             Sum of           Mean
         Source                   DF        Squares         Square    F Value    Pr > F

         Model                     1        4.23757        4.23757     104.51    0.0002
         Error                     5        0.20274        0.04055
         Corrected Total           6        4.44032


                      Root MSE              0.20137    R-Square     0.9543
                      Dependent Mean        0.57541    Adj R-Sq     0.9452
                      Coeff Var            34.99557
```

```
                                      Parameter Estimates

                                   Parameter       Standard
              Variable     DF       Estimate          Error    t Value    Pr > |t|

              Intercept     1        0.47992        0.07668       6.26      0.0015
              LNE           1        1.82057        0.17809      10.22      0.0002

                            FREUNDLICH REGRESSION on TEMPERATURE=35
18
                                                                18:32 Saturday, October
25, 2003

           Obs           Temp               E               A      EbyA       Einv

            1              35           0.001           0.001        .          .
            2              35           0.477            0.51    0.93529    2.09644
            3              35           0.715             0.6    1.19167    1.39860
            4              35           0.929            1.56    0.59551    1.07643
            5              35           1.315            2.66    0.49436    0.76046
            6              35           1.335            3.03    0.44059    0.74906
            7              35           1.463           3.179    0.46021    0.68353
            8              35           1.774            4.59    0.38649    0.56370

           Obs      Ainv        LNE         LNA      CMBLNGE      PRED         RESID

            1        .           .           .          .           .           .
            2     1.96078    -0.74024    -0.67334    1.44791    -0.86773     0.19439
            3     1.66667    -0.33547    -0.51083    1.18262    -0.13083    -0.38000
            4     0.64103    -0.07365     0.44469    1.03751     0.34584     0.09884
            5     0.37594     0.27384     0.97833    0.87204     0.97846    -0.00013
            6     0.33003     0.28893     1.10856    0.86548     1.00594     0.10262
            7     0.31456     0.38049     1.15657    0.82676     1.17263    -0.01606
            8     0.21786     0.57324     1.52388    0.75080     1.52354     0.00034

                         COMBINED LANGMUIR REGRESSION on TEMPERATURE=35
19
                                                                18:32 Saturday, October
25, 2003

                                       The REG Procedure
                                         Model: MODEL1
                                   Dependent Variable: Ainv

                                      Analysis of Variance

                                              Sum of           Mean
         Source                   DF         Squares         Square    F Value    Pr > F

         Model                     1         2.86710        2.86710      62.14    0.0005
         Error                     5         0.23069        0.04614
         Corrected Total           6         3.09779


                      Root MSE              0.21480    R-Square     0.9255
                      Dependent Mean        0.78670    Adj R-Sq     0.9106
                      Coeff Var            27.30381


                                      Parameter Estimates

                                   Parameter       Standard
              Variable     DF       Estimate          Error    t Value    Pr > |t|

              Intercept     1       -2.02110        0.36532      -5.53      0.0026
              CMBLNGE       1        2.81458        0.35705       7.88      0.0005

                         COMBINED LANGMUIR REGRESSION on TEMPERATURE=35
20
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                                                        18:32 Saturday, October
25, 2003

          Obs            Temp               E               A      EbyA       Einv

            1              35           0.001           0.001       .           .
            2              35           0.477            0.51    0.93529    2.09644
            3              35           0.715             0.6    1.19167    1.39860
            4              35           0.929            1.56    0.59551    1.07643
            5              35           1.315            2.66    0.49436    0.76046
            6              35           1.335            3.03    0.44059    0.74906
            7              35           1.463           3.179    0.46021    0.68353
            8              35           1.774            4.59    0.38649    0.56370

          Obs      Ainv        LNE         LNA      CMBLNGE      PRED       RESID

            1        .          .           .          .          .           .
            2     1.96078    -0.74024    -0.67334    1.44791    2.05416    -0.09337
            3     1.66667    -0.33547    -0.51083    1.18262    1.30750     0.35917
            4     0.64103    -0.07365     0.44469    1.03751    0.89906    -0.25803
            5     0.37594     0.27384     0.97833    0.87204    0.43333    -0.05739
            6     0.33003     0.28893     1.10856    0.86548    0.41488    -0.08485
            7     0.31456     0.38049     1.15657    0.82676    0.30588     0.00869
            8     0.21786     0.57324     1.52388    0.75080    0.09208     0.12578

                         A versus E REGRESSION on TEMPERATURE=45
21
                                                        18:32 Saturday, October
25, 2003

                                    The REG Procedure
                                      Model: MODEL1
                                  Dependent Variable: A

                                   Analysis of Variance

                                               Sum of           Mean
        Source                   DF          Squares         Square    F Value    Pr > F

        Model                     1          8.03851        8.03851      43.41    0.0006
        Error                     6          1.11098        0.18516
        Corrected Total           7          9.14949


                     Root MSE              0.43031    R-Square     0.8786
                     Dependent Mean        1.63263    Adj R-Sq     0.8583
                     Coeff Var            26.35665


                                   Parameter Estimates

                                   Parameter       Standard
             Variable     DF        Estimate          Error    t Value    Pr > |t|

             Intercept     1        -0.20621        0.31786      -0.65      0.5405
             E             1         1.83677        0.27877       6.59      0.0006

                         A versus E REGRESSION on TEMPERATURE=45
22
                                                        18:32 Saturday, October
25, 2003

          Obs            Temp               E               A      EbyA       Einv

            1              45           0.001           0.001       .           .
            2              45           0.477            0.46    1.03696    2.09644
            3              45           0.715            0.78    0.91667    1.39860
            4              45           0.929            1.61    0.57702    1.07643
            5              45           1.315            1.83    0.71858    0.76046
            6              45           1.335            2.48    0.53831    0.74906
            7              45           1.463            3.23    0.45294    0.68353
```

```
            8               45           1.774            2.67    0.66442    0.56370

           Obs      Ainv        LNE         LNA      CMBLNGE      PRED        RESID

            1      .           .           .          .         -0.20437     0.20537
            2     2.17391    -0.74024    -0.77653    1.44791     0.66993    -0.20993
            3     1.28205    -0.33547    -0.24846    1.18262     1.10708    -0.32708
            4     0.62112    -0.07365     0.47623    1.03751     1.50015     0.10985
            5     0.54645     0.27384     0.60432    0.87204     2.20914    -0.37914
            6     0.40323     0.28893     0.90826    0.86548     2.24588     0.23412
            7     0.30960     0.38049     1.17248    0.82676     2.48098     0.74902
            8     0.37453     0.57324     0.98208    0.75080     3.05222    -0.38222

                           EbyA versus E REGRESSION on TEMPERATURE=45
23
                                                                18:32 Saturday, October
25, 2003

                                       The REG Procedure
                                         Model: MODEL1
                                   Dependent Variable: EbyA

                                      Analysis of Variance

                                             Sum of           Mean
         Source                   DF        Squares         Square    F Value    Pr > F

         Model                     1        0.14244        0.14244       5.84    0.0604
         Error                     5        0.12196        0.02439
         Corrected Total           6        0.26440


                      Root MSE              0.15618    R-Square     0.5387
                      Dependent Mean        0.70070    Adj R-Sq     0.4465
                      Coeff Var            22.28886


                                      Parameter Estimates

                                   Parameter       Standard
              Variable     DF       Estimate          Error    t Value    Pr > |t|

              Intercept     1        1.08850        0.17099       6.37      0.0014
              E             1       -0.33899        0.14028      -2.42      0.0604

                           EbyA versus E REGRESSION on TEMPERATURE=45
24
                                                                18:32 Saturday, October
25, 2003

           Obs            Temp               E               A      EbyA       Einv

            1               45           0.001           0.001     .          .
            2               45           0.477            0.46    1.03696    2.09644
            3               45           0.715            0.78    0.91667    1.39860
            4               45           0.929            1.61    0.57702    1.07643
            5               45           1.315            1.83    0.71858    0.76046
            6               45           1.335            2.48    0.53831    0.74906
            7               45           1.463            3.23    0.45294    0.68353
            8               45           1.774            2.67    0.66442    0.56370

           Obs      Ainv        LNE         LNA      CMBLNGE      PRED       RESID

            1      .           .           .          .         1.08817      .
            2     2.17391    -0.74024    -0.77653    1.44791    0.92681     0.11015
            3     1.28205    -0.33547    -0.24846    1.18262    0.84613     0.07054
            4     0.62112    -0.07365     0.47623    1.03751    0.77358    -0.19656
            5     0.54645     0.27384     0.60432    0.87204    0.64273     0.07585
            6     0.40323     0.28893     0.90826    0.86548    0.63595    -0.09764
            7     0.30960     0.38049     1.17248    0.82676    0.59256    -0.13962
            8     0.37453     0.57324     0.98208    0.75080    0.48713     0.17729
```

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                        LANGMUIR REGRESSION on TEMPERATURE=45
25
                                                          18:32 Saturday, October
25, 2003

                                   The REG Procedure
                                     Model: MODEL1
                               Dependent Variable: Ainv

                                  Analysis of Variance

                                          Sum of           Mean
         Source                   DF     Squares         Square    F Value    Pr > F

         Model                     1     2.69862        2.69862     142.22    <.0001
         Error                     5     0.09487        0.01897
         Corrected Total           6     2.79349


                      Root MSE              0.13775    R-Square     0.9660
                      Dependent Mean        0.81584    Adj R-Sq     0.9592
                      Coeff Var            16.88429


                                  Parameter Estimates

                                    Parameter       Standard
              Variable     DF       Estimate          Error    t Value    Pr > |t|

              Intercept     1       -0.47961        0.12046      -3.98      0.0105
              Einv          1        1.23743        0.10376      11.93      <.0001
                        LANGMUIR REGRESSION on TEMPERATURE=45
26
                                                          18:32 Saturday, October
25, 2003

           Obs           Temp              E              A      EbyA       Einv

            1              45          0.001          0.001       .          .
            2              45          0.477           0.46    1.03696    2.09644
            3              45          0.715           0.78    0.91667    1.39860
            4              45          0.929           1.61    0.57702    1.07643
            5              45          1.315           1.83    0.71858    0.76046
            6              45          1.335           2.48    0.53831    0.74906
            7              45          1.463           3.23    0.45294    0.68353
            8              45          1.774           2.67    0.66442    0.56370

           Obs      Ainv         LNE          LNA      CMBLNGE      PRED       RESID

            1      .           .            .           .          .          .
            2     2.17391    -0.74024    -0.77653    1.44791    2.11459     0.05933
            3     1.28205    -0.33547    -0.24846    1.18262    1.25106     0.03099
            4     0.62112    -0.07365     0.47623    1.03751    0.85239    -0.23128
            5     0.54645     0.27384     0.60432    0.87204    0.46140     0.08505
            6     0.40323     0.28893     0.90826    0.86548    0.44730    -0.04408
            7     0.30960     0.38049     1.17248    0.82676    0.36621    -0.05661
            8     0.37453     0.57324     0.98208    0.75080    0.21793     0.15661
                       FREUNDLICH REGRESSION on TEMPERATURE=45
27
                                                          18:32 Saturday, October
25, 2003

                                   The REG Procedure
                                     Model: MODEL1
                                Dependent Variable: LNA

                                  Analysis of Variance
```

```
                                             Sum of           Mean
         Source                   DF        Squares         Square    F Value    Pr > F

         Model                     1        2.81331        2.81331      64.42    0.0005
         Error                     5        0.21836        0.04367
         Corrected Total           6        3.03167


                      Root MSE              0.20898    R-Square     0.9280
                      Dependent Mean        0.44548    Adj R-Sq     0.9136
                      Coeff Var            46.91012


                                      Parameter Estimates

                                   Parameter       Standard
              Variable     DF       Estimate          Error    t Value    Pr > |t|

              Intercept     1        0.36768        0.07958       4.62      0.0057
              LNE           1        1.48340        0.18482       8.03      0.0005

                            FREUNDLICH REGRESSION on TEMPERATURE=45
28
                                                                18:32 Saturday, October
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           Obs           Temp              E              A      EbyA       Einv

            1             45           0.001          0.001     .          .
            2             45           0.477           0.46    1.03696    2.09644
            3             45           0.715           0.78    0.91667    1.39860
            4             45           0.929           1.61    0.57702    1.07643
            5             45           1.315           1.83    0.71858    0.76046
            6             45           1.335           2.48    0.53831    0.74906
            7             45           1.463           3.23    0.45294    0.68353
            8             45           1.774           2.67    0.66442    0.56370

           Obs      Ainv        LNE         LNA      CMBLNGE      PRED        RESID

            1       .          .           .          .           .           .
            2     2.17391    -0.74024    -0.77653    1.44791    -0.73039    -0.04614
            3     1.28205    -0.33547    -0.24846    1.18262    -0.12996    -0.11850
            4     0.62112    -0.07365     0.47623    1.03751     0.25843     0.21780
            5     0.54645     0.27384     0.60432    0.87204     0.77389    -0.16957
            6     0.40323     0.28893     0.90826    0.86548     0.79628     0.11198
            7     0.30960     0.38049     1.17248    0.82676     0.93210     0.24038
            8     0.37453     0.57324     0.98208    0.75080     1.21802    -0.23594

                         COMBINED LANGMUIR REGRESSION on TEMPERATURE=45
29
                                                                18:32 Saturday, October
25, 2003

                                       The REG Procedure
                                         Model: MODEL1
                                   Dependent Variable: Ainv

                                      Analysis of Variance

                                             Sum of           Mean
         Source                   DF        Squares         Square    F Value    Pr > F

         Model                     1        2.62004        2.62004      75.53    0.0003
         Error                     5        0.17345        0.03469
         Corrected Total           6        2.79349


                      Root MSE              0.18625    R-Square     0.9379
                      Dependent Mean        0.81584    Adj R-Sq     0.9255
                      Coeff Var            22.82955
```

```
                              Parameter Estimates

                             Parameter       Standard
         Variable     DF       Estimate          Error    t Value    Pr > |t|

         Intercept     1       -1.86826        0.31677      -5.90      0.0020
         CMBLNGE       1        2.69058        0.30960       8.69      0.0003

                     COMBINED LANGMUIR REGRESSION on TEMPERATURE=45
30
                                                           18:32 Saturday, October
25, 2003

            Obs           Temp              E              A      EbyA       Einv

              1             45          0.001          0.001      .          .
              2             45          0.477           0.46    1.03696    2.09644
              3             45          0.715           0.78    0.91667    1.39860
              4             45          0.929           1.61    0.57702    1.07643
              5             45          1.315           1.83    0.71858    0.76046
              6             45          1.335           2.48    0.53831    0.74906
              7             45          1.463           3.23    0.45294    0.68353
              8             45          1.774           2.67    0.66442    0.56370

            Obs      Ainv        LNE         LNA      CMBLNGE      PRED       RESID

              1      .          .           .          .          .          .
              2    2.17391    -0.74024    -0.77653    1.44791    2.02746     0.14645
              3    1.28205    -0.33547    -0.24846    1.18262    1.31370    -0.03164
              4    0.62112    -0.07365     0.47623    1.03751    0.92325    -0.30213
              5    0.54645     0.27384     0.60432    0.87204    0.47804     0.06840
              6    0.40323     0.28893     0.90826    0.86548    0.46040    -0.05718
              7    0.30960     0.38049     1.17248    0.82676    0.35620    -0.04661
              8    0.37453     0.57324     0.98208    0.75080    0.15183     0.22270

                        A versus E REGRESSION on TEMPERATURE=55
31
                                                           18:32 Saturday, October
25, 2003

                               The REG Procedure
                                 Model: MODEL1
                             Dependent Variable: A

                              Analysis of Variance

                                        Sum of           Mean
       Source                   DF         Squares         Square    F Value    Pr > F

       Model                     1        33.28316       33.28316     210.93    <.0001
       Error                     6         0.94677        0.15780
       Corrected Total           7        34.22993


                    Root MSE              0.39724    R-Square     0.9723
                    Dependent Mean        3.57888    Adj R-Sq     0.9677
                    Coeff Var            11.09944


                              Parameter Estimates

                             Parameter       Standard
         Variable     DF       Estimate          Error    t Value    Pr > |t|

         Intercept     1       -0.16281        0.29343      -0.55      0.5990
         E             1        3.73748        0.25734      14.52      <.0001

                        A versus E REGRESSION on TEMPERATURE=55
32
                                                           18:32 Saturday, October
```

```
25, 2003

           Obs            Temp               E               A      EbyA       Einv

            1               55           0.001           0.001     .          .
            2               55           0.477            1.03    0.46311    2.09644
            3               55           0.715            2.39    0.29916    1.39860
            4               55           0.929            3.93    0.23639    1.07643
            5               55           1.315            4.63    0.28402    0.76046
            6               55           1.335            5.07    0.26331    0.74906
            7               55           1.463            5.41    0.27043    0.68353
            8               55           1.774            6.17    0.28752    0.56370

           Obs      Ainv        LNE        LNA      CMBLNGE      PRED        RESID

            1      .           .          .          .         -0.15908     0.16008
            2     0.97087    -0.74024    0.02956    1.44791     1.61997    -0.58997
            3     0.41841    -0.33547    0.87129    1.18262     2.50949    -0.11949
            4     0.25445    -0.07365    1.36864    1.03751     3.30931     0.62069
            5     0.21598     0.27384    1.53256    0.87204     4.75198    -0.12198
            6     0.19724     0.28893    1.62334    0.86548     4.82673     0.24327
            7     0.18484     0.38049    1.68825    0.82676     5.30513     0.10487
            8     0.16207     0.57324    1.81970    0.75080     6.46748    -0.29748

                           EbyA versus E REGRESSION on TEMPERATURE=55
33
                                                                18:32 Saturday, October
25, 2003

                                       The REG Procedure
                                         Model: MODEL1
                                   Dependent Variable: EbyA

                                      Analysis of Variance

                                             Sum of           Mean
         Source                    DF         Squares         Square    F Value    Pr > F

         Model                      1         0.01197        0.01197       2.81    0.1547
         Error                      5         0.02131        0.00426
         Corrected Total            6         0.03328


                      Root MSE              0.06529    R-Square     0.3596
                      Dependent Mean        0.30056    Adj R-Sq     0.2315
                      Coeff Var            21.72254


                                      Parameter Estimates

                                   Parameter       Standard
              Variable     DF       Estimate          Error    t Value    Pr > |t|

              Intercept     1        0.41297        0.07148       5.78      0.0022
              E             1       -0.09826        0.05864      -1.68      0.1547

                           EbyA versus E REGRESSION on TEMPERATURE=55
34
                                                                18:32 Saturday, October
25, 2003

           Obs            Temp               E               A      EbyA       Einv

            1               55           0.001           0.001     .          .
            2               55           0.477            1.03    0.46311    2.09644
            3               55           0.715            2.39    0.29916    1.39860
            4               55           0.929            3.93    0.23639    1.07643
            5               55           1.315            4.63    0.28402    0.76046
            6               55           1.335            5.07    0.26331    0.74906
            7               55           1.463            5.41    0.27043    0.68353
            8               55           1.774            6.17    0.28752    0.56370
```

```
           Obs      Ainv        LNE        LNA      CMBLNGE      PRED       RESID

            1      .           .          .          .       0.41287       .
            2     0.97087    -0.74024    0.02956    1.44791    0.36610     0.097008
            3     0.41841    -0.33547    0.87129    1.18262    0.34271    -0.043551
            4     0.25445    -0.07365    1.36864    1.03751    0.32169    -0.085300
            5     0.21598     0.27384    1.53256    0.87204    0.28376     0.000257
            6     0.19724     0.28893    1.62334    0.86548    0.28179    -0.018481
            7     0.18484     0.38049    1.68825    0.82676    0.26922     0.001207
            8     0.16207     0.57324    1.81970    0.75080    0.23866     0.048860

                             LANGMUIR REGRESSION on TEMPERATURE=55
35
                                                                18:32 Saturday, October
25, 2003

                                       The REG Procedure
                                         Model: MODEL1
                                   Dependent Variable: Ainv

                                      Analysis of Variance

                                             Sum of           Mean
         Source                   DF        Squares         Square    F Value    Pr > F

         Model                     1        0.46738        0.46738      65.82    0.0005
         Error                     5        0.03550        0.00710
         Corrected Total           6        0.50288


                      Root MSE              0.08427    R-Square     0.9294
                      Dependent Mean        0.34341    Adj R-Sq     0.9153
                      Coeff Var            24.53787


                                      Parameter Estimates

                                   Parameter       Standard
              Variable     DF       Estimate          Error    t Value    Pr > |t|

              Intercept     1       -0.19571        0.07369      -2.66      0.0451
              Einv          1        0.51497        0.06347       8.11      0.0005

                             LANGMUIR REGRESSION on TEMPERATURE=55
36
                                                                18:32 Saturday, October
25, 2003

           Obs           Temp               E               A      EbyA       Einv

            1              55           0.001           0.001     .          .
            2              55           0.477            1.03    0.46311    2.09644
            3              55           0.715            2.39    0.29916    1.39860
            4              55           0.929            3.93    0.23639    1.07643
            5              55           1.315            4.63    0.28402    0.76046
            6              55           1.335            5.07    0.26331    0.74906
            7              55           1.463            5.41    0.27043    0.68353
            8              55           1.774            6.17    0.28752    0.56370

           Obs      Ainv        LNE        LNA      CMBLNGE      PRED       RESID

            1      .           .          .          .          .          .
            2     0.97087    -0.74024    0.02956    1.44791    0.88390     0.08697
            3     0.41841    -0.33547    0.87129    1.18262    0.52453    -0.10612
            4     0.25445    -0.07365    1.36864    1.03751    0.35862    -0.10417
            5     0.21598     0.27384    1.53256    0.87204    0.19591     0.02008
            6     0.19724     0.28893    1.62334    0.86548    0.19004     0.00720
            7     0.18484     0.38049    1.68825    0.82676    0.15629     0.02855
            8     0.16207     0.57324    1.81970    0.75080    0.09458     0.06749
```

```
                        FREUNDLICH REGRESSION on TEMPERATURE=55
37
                                                              18:32 Saturday, October
25, 2003

                                   The REG Procedure
                                     Model: MODEL1
                                Dependent Variable: LNA

                                  Analysis of Variance

                                         Sum of           Mean
         Source                   DF        Squares         Square    F Value    Pr > F

         Model                     1        2.23573        2.23573      78.57    0.0003
         Error                     5        0.14228        0.02846
         Corrected Total           6        2.37801


                      Root MSE              0.16869    R-Square     0.9402
                      Dependent Mean        1.27619    Adj R-Sq     0.9282
                      Coeff Var            13.21828


                                  Parameter Estimates

                                  Parameter       Standard
              Variable     DF      Estimate          Error    t Value    Pr > |t|

              Intercept     1       1.20683        0.06424      18.79      <.0001
              LNE           1       1.32239        0.14919       8.86      0.0003

                        FREUNDLICH REGRESSION on TEMPERATURE=55
38
                                                              18:32 Saturday, October
25, 2003

           Obs           Temp              E              A      EbyA       Einv

            1             55           0.001          0.001        .          .
            2             55           0.477           1.03    0.46311    2.09644
            3             55           0.715           2.39    0.29916    1.39860
            4             55           0.929           3.93    0.23639    1.07643
            5             55           1.315           4.63    0.28402    0.76046
            6             55           1.335           5.07    0.26331    0.74906
            7             55           1.463           5.41    0.27043    0.68353
            8             55           1.774           6.17    0.28752    0.56370

           Obs      Ainv        LNE        LNA      CMBLNGE      PRED       RESID

            1         .          .          .          .          .          .
            2     0.97087   -0.74024    0.02956    1.44791    0.22795    -0.19839
            3     0.41841   -0.33547    0.87129    1.18262    0.76321     0.10808
            4     0.25445   -0.07365    1.36864    1.03751    1.10945     0.25919
            5     0.21598    0.27384    1.53256    0.87204    1.56895    -0.03640
            6     0.19724    0.28893    1.62334    0.86548    1.58891     0.03443
            7     0.18484    0.38049    1.68825    0.82676    1.70999    -0.02174
            8     0.16207    0.57324    1.81970    0.75080    1.96488    -0.14518

                     COMBINED LANGMUIR REGRESSION on TEMPERATURE=55
39
                                                              18:32 Saturday, October
25, 2003

                                   The REG Procedure
                                     Model: MODEL1
                                Dependent Variable: Ainv

                                  Analysis of Variance

                                         Sum of           Mean
```

```
         Source                   DF       Squares         Square    F Value    Pr > F

         Model                     1       0.44216        0.44216      36.41    0.0018
         Error                     5       0.06072        0.01214
         Corrected Total           6       0.50288


                      Root MSE              0.11020    R-Square     0.8793
                      Dependent Mean        0.34341    Adj R-Sq     0.8551
                      Coeff Var            32.08999


                                      Parameter Estimates

                                   Parameter       Standard
              Variable     DF       Estimate          Error    t Value    Pr > |t|

              Intercept     1       -0.75923        0.18742      -4.05      0.0098
              CMBLNGE       1        1.10530        0.18318       6.03      0.0018

                         COMBINED LANGMUIR REGRESSION on TEMPERATURE=55
40
                                                                18:32 Saturday, October
25, 2003

           Obs           Temp             E              A      EbyA       Einv

            1              55         0.001          0.001        .          .
            2              55         0.477           1.03    0.46311    2.09644
            3              55         0.715           2.39    0.29916    1.39860
            4              55         0.929           3.93    0.23639    1.07643
            5              55         1.315           4.63    0.28402    0.76046
            6              55         1.335           5.07    0.26331    0.74906
            7              55         1.463           5.41    0.27043    0.68353
            8              55         1.774           6.17    0.28752    0.56370

           Obs      Ainv        LNE         LNA      CMBLNGE      PRED       RESID

            1        .           .           .          .           .          .
            2     0.97087    -0.74024    0.02956    1.44791    0.84115     0.12972
            3     0.41841    -0.33547    0.87129    1.18262    0.54793    -0.12952
            4     0.25445    -0.07365    1.36864    1.03751    0.38754    -0.13308
            5     0.21598     0.27384    1.53256    0.87204    0.20464     0.01134
            6     0.19724     0.28893    1.62334    0.86548    0.19740    -0.00016
            7     0.18484     0.38049    1.68825    0.82676    0.15459     0.03025
            8     0.16207     0.57324    1.81970    0.75080    0.07063     0.09144
```

Appendix C

Statistical Analysis for Experimental Adsorption Data of EG II to fit Adsorption Models

SAS CODE

```
%let path=D:\Dole Files\;
libname bus "&path.";
/* THIS SAS FILE IS FOR REGRESSION FOR DATA ON EGIIa FILE */

/* This step makes new variables for calculating different models */

data temp;
set bus.egIIa;
if E>0.001 then do;
m=2;
EbyA=E/A;
Einv=1/E;
Ainv=1/A;
LNE=log(E);
LNA=log(A);
CMBLNGE=1/(E**(1/m));
drop m;
end;
run;


/**********************************************/
/* regression models ..... FOR TEMPERATURE=25 */
/**********************************************/

/* Selecting Cases */

data temp25;
set temp;
if TEMP=25 then output;
run;

/* A versus E Regression */
title 'A versus E REGRESSION on TEMPERATURE=25';
PROC REG data=temp25;
  MODEL A=E;
OUTPUT OUT=AE P=PRED R=RESID;
PLOT A*E='*' PREDICTED.*E='o' RESIDUAL.*E='x';
PROC PRINT DATA=AE;
run;

/* EbyA versus E Regression */
title 'EbyA versus E REGRESSION on TEMPERATURE=25';
PROC REG data=temp25;
  MODEL EbyA=E;
OUTPUT OUT=EbyA P=PRED R=RESID;
PLOT EbyA*E='*' PREDICTED.*E='o' RESIDUAL.*E='x';
PROC PRINT DATA=EbyA;
run;


/* Langmuir Regression */
title 'LANGMUIR REGRESSION on TEMPERATURE=25';
PROC REG data=temp25;
  MODEL Ainv=Einv;
```

```
OUTPUT OUT=LANGMUIR P=PRED R=RESID;
PLOT Ainv*Einv='*' PREDICTED.*Einv='o' RESIDUAL.*Einv='x';
PROC PRINT DATA=LANGMUIR;
run;

/* Freundlich Regression */
title 'FREUNDLICH REGRESSION on TEMPERATURE=25';
PROC REG data=temp25;
MODEL LNA=LNE;
OUTPUT OUT=FREUNDLICH P=PRED R=RESID;
PLOT LNA*LNE='*' PREDICTED.*LNE='o' RESIDUAL.*LNE='x';
PROC PRINT DATA=FREUNDLICH;
run;

/* Combined Langmuir Regression */
title 'COMBINED LANGMUIR REGRESSION on TEMPERATURE=25';
PROC REG data=temp25;
MODEL Ainv=CMBLNGE;
OUTPUT OUT=COMBLANGMUIR P=PRED R=RESID;
PLOT Ainv*CMBLNGE='*' PREDICTED.*CMBLNGE='o' RESIDUAL.*CMBLNGE='x';
PROC PRINT DATA=COMBLANGMUIR;
run;


/*********************************************/
/* regression models ..... FOR TEMPERATURE=35 */
/*********************************************/

/* Selecting Cases */

data temp35;
set temp;
if TEMP=35 then output;
run;

/* A versus E Regression */
title 'A versus E REGRESSION on TEMPERATURE=35';
PROC REG data=temp35;
  MODEL A=E;
OUTPUT OUT=AE P=PRED R=RESID;
PLOT A*E='*' PREDICTED.*E='o' RESIDUAL.*E='x';
PROC PRINT DATA=AE;
run;


/* EbyA versus E Regression */
title 'EbyA versus E REGRESSION on TEMPERATURE=35';
PROC REG data=temp35;
  MODEL EbyA=E;
OUTPUT OUT=EbyA P=PRED R=RESID;
PLOT EbyA*E='*' PREDICTED.*E='o' RESIDUAL.*E='x';
PROC PRINT DATA=EbyA;
run;

/* Langmuir Regression */
title 'LANGMUIR REGRESSION on TEMPERATURE=35';
PROC REG data=temp35;
```

```
  MODEL Ainv=Einv;
OUTPUT OUT=LANGMUIR P=PRED R=RESID;
PLOT Ainv*Einv='*' PREDICTED.*Einv='o' RESIDUAL.*Einv='x';
PROC PRINT DATA=LANGMUIR;
run;

/* Freundlich Regression */
title 'FREUNDLICH REGRESSION on TEMPERATURE=35';
PROC REG data=temp35;
MODEL LNA=LNE;
OUTPUT OUT=FREUNDLICH P=PRED R=RESID;
PLOT LNA*LNE='*' PREDICTED.*LNE='o' RESIDUAL.*LNE='x';
PROC PRINT DATA=FREUNDLICH;
run;

/* Combined Langmuir Regression */
title 'COMBINED LANGMUIR REGRESSION on TEMPERATURE=35';
PROC REG data=temp35;
MODEL Ainv=CMBLNGE;
OUTPUT OUT=COMBLANGMUIR P=PRED R=RESID;
PLOT Ainv*CMBLNGE='*' PREDICTED.*CMBLNGE='o' RESIDUAL.*CMBLNGE='x';
PROC PRINT DATA=COMBLANGMUIR;
run;


/**********************************************/
/* regression models ..... FOR TEMPERATURE=45 */
/**********************************************/

/* Selecting Cases */

data temp45;
set temp;
if TEMP=45 then output;
run;

/* A versus E Regression */
title 'A versus E REGRESSION on TEMPERATURE=45';
PROC REG data=temp45;
  MODEL A=E;
OUTPUT OUT=AE P=PRED R=RESID;
PLOT A*E='*' PREDICTED.*E='o' RESIDUAL.*E='x';
PROC PRINT DATA=AE;
run;

/* EbyA versus E Regression */
title 'EbyA versus E REGRESSION on TEMPERATURE=45';
PROC REG data=temp45;
  MODEL EbyA=E;
OUTPUT OUT=EbyA P=PRED R=RESID;
PLOT EbyA*E='*' PREDICTED.*E='o' RESIDUAL.*E='x';
PROC PRINT DATA=EbyA;
run;

/* Langmuir Regression */
title 'LANGMUIR REGRESSION on TEMPERATURE=45';
PROC REG data=temp45;
```

```
  MODEL Ainv=Einv;
OUTPUT OUT=LANGMUIR P=PRED R=RESID;
PLOT Ainv*Einv='*' PREDICTED.*Einv='o' RESIDUAL.*Einv='x';
PROC PRINT DATA=LANGMUIR;
run;

/* Freundlich Regression */
title 'FREUNDLICH REGRESSION on TEMPERATURE=45';
PROC REG data=temp45;
MODEL LNA=LNE;
OUTPUT OUT=FREUNDLICH P=PRED R=RESID;
PLOT LNA*LNE='*' PREDICTED.*LNE='o' RESIDUAL.*LNE='x';
PROC PRINT DATA=FREUNDLICH;
run;

/* Combined Langmuir Regression */
title 'COMBINED LANGMUIR REGRESSION on TEMPERATURE=45';
PROC REG data=temp45;
MODEL Ainv=CMBLNGE;
OUTPUT OUT=COMBLANGMUIR P=PRED R=RESID;
PLOT Ainv*CMBLNGE='*' PREDICTED.*CMBLNGE='o' RESIDUAL.*CMBLNGE='x';
PROC PRINT DATA=COMBLANGMUIR;
run;


/*********************************************/
/* regression models ..... FOR TEMPERATURE=55 */
/*********************************************/

/* Selecting Cases */

data temp55;
set temp;
if TEMP=55 then output;
run;

/* A versus E Regression */
title 'A versus E REGRESSION on TEMPERATURE=55';
PROC REG data=temp55;
  MODEL A=E;
OUTPUT OUT=AE P=PRED R=RESID;
PLOT A*E='*' PREDICTED.*E='o' RESIDUAL.*E='x';
PROC PRINT DATA=AE;
run;


/* EbyA versus E Regression */
title 'EbyA versus E REGRESSION on TEMPERATURE=55';
PROC REG data=temp55;
  MODEL EbyA=E;
OUTPUT OUT=EbyA P=PRED R=RESID;
PLOT EbyA*E='*' PREDICTED.*E='o' RESIDUAL.*E='x';
PROC PRINT DATA=EbyA;
run;


/* Langmuir Regression */
```

```
title 'LANGMUIR REGRESSION on TEMPERATURE=55';
PROC REG data=temp55;
  MODEL Ainv=Einv;
OUTPUT OUT=LANGMUIR P=PRED R=RESID;
PLOT Ainv*Einv='*' PREDICTED.*Einv='o' RESIDUAL.*Einv='x';
PROC PRINT DATA=LANGMUIR;
run;

/* Freundlich Regression */
title 'FREUNDLICH REGRESSION on TEMPERATURE=55';
PROC REG data=temp55;
MODEL LNA=LNE;
OUTPUT OUT=FREUNDLICH P=PRED R=RESID;
PLOT LNA*LNE='*' PREDICTED.*LNE='o' RESIDUAL.*LNE='x';
PROC PRINT DATA=FREUNDLICH;
run;

/* Combined Langmuir Regression */
title 'COMBINED LANGMUIR REGRESSION on TEMPERATURE=55';
PROC REG data=temp55;
MODEL Ainv=CMBLNGE;
OUTPUT OUT=COMBLANGMUIR P=PRED R=RESID;
PLOT Ainv*CMBLNGE='*' PREDICTED.*CMBLNGE='o' RESIDUAL.*CMBLNGE='x';
PROC PRINT DATA=COMBLANGMUIR;
run;
```

SAS OUTPUT

```
                            A versus E REGRESSION on TEMPERATURE=25
1
                                                                18:38 Saturday, October
25, 2003

                                       The REG Procedure
                                         Model: MODEL1
                                     Dependent Variable: A

                                      Analysis of Variance

                                             Sum of           Mean
         Source                   DF        Squares         Square    F Value    Pr > F

         Model                     1        9.55896        9.55896     140.83    <.0001
         Error                     6        0.40724        0.06787
         Corrected Total           7        9.96620


                      Root MSE              0.26053    R-Square     0.9591
                      Dependent Mean        1.68526    Adj R-Sq     0.9523
                      Coeff Var            15.45907


                                      Parameter Estimates

                                   Parameter       Standard
              Variable     DF       Estimate          Error    t Value    Pr > |t|

              Intercept     1       -0.06860        0.17414      -0.39      0.7072
              E             1        1.61805        0.13634      11.87      <.0001

                            A versus E REGRESSION on TEMPERATURE=25
2
                                                                18:38 Saturday, October
25, 2003

           Obs           Temp                E               A       EbyA       Einv

            1             25             0.001           0.001     .          .
            2             25            0.3619            0.21    1.72333    2.76319
            3             25            0.6896            1.03    0.66951    1.45012
            4             25             0.997            1.93    0.51658    1.00301
            5             25              1.14            1.62    0.70370    0.87719
            6             25             1.556            2.71    0.57417    0.64267
            7             25             1.857            2.96    0.62736    0.53850
            8             25             2.069     3.021111111    0.68485    0.48333

           Obs      Ainv        LNE         LNA      CMBLNGE      PRED         RESID

            1      .           .           .          .         -0.06699     0.06799
            2     4.76190    -1.01639    -1.56065    1.66229     0.51697    -0.30697
            3     0.97087    -0.37164     0.02956    1.20421     1.04721    -0.01721
            4     0.51813    -0.00300     0.65752    1.00150     1.54459     0.38541
            5     0.61728     0.13103     0.48243    0.93659     1.77598    -0.15598
            6     0.36900     0.44212     0.99695    0.80167     2.44909     0.26091
            7     0.33784     0.61896     1.08519    0.73383     2.93612     0.02388
            8     0.33100     0.72707     1.10562    0.69522     3.27915    -0.25804

                           EbyA versus E REGRESSION on TEMPERATURE=25
3
                                                                18:38 Saturday, October
25, 2003

                                       The REG Procedure
                                         Model: MODEL1
                                   Dependent Variable: EbyA
```

```
                              Analysis of Variance

                                      Sum of           Mean
        Source                 DF        Squares         Square    F Value    Pr > F

        Model                   1        0.37746        0.37746       2.80    0.1552
        Error                   5        0.67433        0.13487
        Corrected Total         6        1.05179


                     Root MSE              0.36724    R-Square     0.3589
                     Dependent Mean        0.78564    Adj R-Sq     0.2306
                     Coeff Var            46.74403


                              Parameter Estimates

                             Parameter       Standard
             Variable     DF       Estimate          Error    t Value    Pr > |t|

             Intercept     1        1.28625        0.32986       3.90      0.0114
             E             1       -0.40416        0.24158      -1.67      0.1552

                         EbyA versus E REGRESSION on TEMPERATURE=25
4
                                                                  18:38 Saturday, October
25, 2003

          Obs           Temp              E              A      EbyA       Einv

           1             25           0.001          0.001       .          .
           2             25          0.3619           0.21    1.72333    2.76319
           3             25          0.6896           1.03    0.66951    1.45012
           4             25           0.997           1.93    0.51658    1.00301
           5             25            1.14           1.62    0.70370    0.87719
           6             25           1.556           2.71    0.57417    0.64267
           7             25           1.857           2.96    0.62736    0.53850
           8             25           2.069    3.021111111    0.68485    0.48333

          Obs      Ainv        LNE          LNA      CMBLNGE       PRED       RESID

           1        .           .            .          .        1.28585       .
           2     4.76190    -1.01639    -1.56065    1.66229    1.13999     0.58335
           3     0.97087    -0.37164     0.02956    1.20421    1.00754    -0.33803
           4     0.51813    -0.00300     0.65752    1.00150    0.88331    -0.36673
           5     0.61728     0.13103     0.48243    0.93659    0.82551    -0.12181
           6     0.36900     0.44212     0.99695    0.80167    0.65738    -0.08321
           7     0.33784     0.61896     1.08519    0.73383    0.53573     0.09163
           8     0.33100     0.72707     1.10562    0.69522    0.45005     0.23480

                           LANGMUIR REGRESSION on TEMPERATURE=25
5
                                                                  18:38 Saturday, October
25, 2003

                                The REG Procedure
                                  Model: MODEL1
                             Dependent Variable: Ainv

                              Analysis of Variance

                                      Sum of           Mean
        Source                 DF        Squares         Square    F Value    Pr > F

        Model                   1       14.38528       14.38528      54.78    0.0007
        Error                   5        1.31306        0.26261
        Corrected Total         6       15.69834


                     Root MSE              0.51246    R-Square     0.9164
                     Dependent Mean        1.12943    Adj R-Sq     0.8996
```

```
                      Coeff Var            45.37291


                                      Parameter Estimates

                                   Parameter       Standard
              Variable     DF       Estimate          Error    t Value    Pr > |t|

              Intercept     1       -1.01229        0.34822      -2.91      0.0335
              Einv          1        1.93246        0.26110       7.40      0.0007

                             LANGMUIR REGRESSION on TEMPERATURE=25
6
                                                                18:38 Saturday, October
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           Obs            Temp               E               A      EbyA       Einv

            1               25           0.001           0.001     .          .
            2               25          0.3619            0.21    1.72333    2.76319
            3               25          0.6896            1.03    0.66951    1.45012
            4               25           0.997            1.93    0.51658    1.00301
            5               25            1.14            1.62    0.70370    0.87719
            6               25           1.556            2.71    0.57417    0.64267
            7               25           1.857            2.96    0.62736    0.53850
            8               25           2.069     3.021111111    0.68485    0.48333

           Obs      Ainv        LNE         LNA      CMBLNGE      PRED        RESID

            1      .           .           .          .           .           .
            2     4.76190    -1.01639    -1.56065    1.66229     4.32748     0.43442
            3     0.97087    -0.37164     0.02956    1.20421     1.79001    -0.81913
            4     0.51813    -0.00300     0.65752    1.00150     0.92599    -0.40785
            5     0.61728     0.13103     0.48243    0.93659     0.68285    -0.06557
            6     0.36900     0.44212     0.99695    0.80167     0.22965     0.13935
            7     0.33784     0.61896     1.08519    0.73383     0.02835     0.30949
            8     0.33100     0.72707     1.10562    0.69522    -0.07828     0.40929

                            FREUNDLICH REGRESSION on TEMPERATURE=25
7
                                                                18:38 Saturday, October
25, 2003

                                       The REG Procedure
                                         Model: MODEL1
                                    Dependent Variable: LNA

                                      Analysis of Variance

                                             Sum of           Mean
         Source                   DF        Squares         Square    F Value    Pr > F

         Model                     1        4.95083        4.95083      57.92    0.0006
         Error                     5        0.42738        0.08548
         Corrected Total           6        5.37821


                      Root MSE              0.29236    R-Square     0.9205
                      Dependent Mean        0.39952    Adj R-Sq     0.9046
                      Coeff Var            73.17906


                                      Parameter Estimates

                                   Parameter       Standard
              Variable     DF       Estimate          Error    t Value    Pr > |t|

              Intercept     1        0.28774        0.11147       2.58      0.0494
              LNE           1        1.48149        0.19466       7.61      0.0006

                            FREUNDLICH REGRESSION on TEMPERATURE=25
```

```
8
                                                                18:38 Saturday, October
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           Obs            Temp               E               A      EbyA       Einv

            1               25           0.001           0.001     .          .
            2               25          0.3619            0.21    1.72333    2.76319
            3               25          0.6896            1.03    0.66951    1.45012
            4               25           0.997            1.93    0.51658    1.00301
            5               25            1.14            1.62    0.70370    0.87719
            6               25           1.556            2.71    0.57417    0.64267
            7               25           1.857            2.96    0.62736    0.53850
            8               25           2.069     3.021111111    0.68485    0.48333

           Obs      Ainv        LNE         LNA      CMBLNGE       PRED        RESID

            1      .          .           .          .          .           .
            2     4.76190    -1.01639    -1.56065    1.66229    -1.21803    -0.34262
            3     0.97087    -0.37164     0.02956    1.20421    -0.26284     0.29240
            4     0.51813    -0.00300     0.65752    1.00150     0.28329     0.37423
            5     0.61728     0.13103     0.48243    0.93659     0.48186     0.00057
            6     0.36900     0.44212     0.99695    0.80167     0.94273     0.05421
            7     0.33784     0.61896     1.08519    0.73383     1.20473    -0.11954
            8     0.33100     0.72707     1.10562    0.69522     1.36488    -0.25926

                         COMBINED LANGMUIR REGRESSION on TEMPERATURE=25
9
                                                                18:38 Saturday, October
25, 2003

                                       The REG Procedure
                                         Model: MODEL1
                                   Dependent Variable: Ainv

                                      Analysis of Variance

                                             Sum of           Mean
         Source                   DF        Squares         Square    F Value    Pr > F

         Model                     1       13.18129       13.18129      26.18    0.0037
         Error                     5        2.51705        0.50341
         Corrected Total           6       15.69834


                      Root MSE              0.70951    R-Square     0.8397
                      Dependent Mean        1.12943    Adj R-Sq     0.8076
                      Coeff Var            62.82030


                                      Parameter Estimates

                                   Parameter       Standard
              Variable     DF       Estimate          Error    t Value    Pr > |t|

              Intercept     1       -3.27213        0.90101      -3.63      0.0150
              CMBLNGE       1        4.37949        0.85587       5.12      0.0037

                         COMBINED LANGMUIR REGRESSION on TEMPERATURE=25
10
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           Obs            Temp               E               A      EbyA       Einv

            1               25           0.001           0.001     .          .
            2               25          0.3619            0.21    1.72333    2.76319
            3               25          0.6896            1.03    0.66951    1.45012
            4               25           0.997            1.93    0.51658    1.00301
            5               25            1.14            1.62    0.70370    0.87719
            6               25           1.556            2.71    0.57417    0.64267
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           7            25          1.857          2.96    0.62736    0.53850
           8            25          2.069   3.021111111    0.68485    0.48333

         Obs      Ainv        LNE          LNA      CMBLNGE      PRED        RESID

           1       .           .            .          .           .           .
           2    4.76190    -1.01639    -1.56065    1.66229     4.00782     0.75408
           3    0.97087    -0.37164     0.02956    1.20421     2.00168    -1.03080
           4    0.51813    -0.00300     0.65752    1.00150     1.11394    -0.59580
           5    0.61728     0.13103     0.48243    0.93659     0.82963    -0.21235
           6    0.36900     0.44212     0.99695    0.80167     0.23877     0.13024
           7    0.33784     0.61896     1.08519    0.73383    -0.05835     0.39618
           8    0.33100     0.72707     1.10562    0.69522    -0.22744     0.55845

                        A versus E REGRESSION on TEMPERATURE=35
11
                                                           18:38 Saturday, October
25, 2003

                                   The REG Procedure
                                    Model: MODEL1
                                 Dependent Variable: A

                                 Analysis of Variance

                                          Sum of           Mean
        Source                   DF        Squares         Square    F Value    Pr > F

        Model                     1       10.70670       10.70670      83.44    <.0001
        Error                     6        0.76992        0.12832
        Corrected Total           7       11.47662


                     Root MSE              0.35822    R-Square     0.9329
                     Dependent Mean        1.76776    Adj R-Sq     0.9217
                     Coeff Var            20.26387


                                  Parameter Estimates

                                 Parameter       Standard
             Variable     DF      Estimate          Error    t Value    Pr > |t|

             Intercept     1      -0.08841        0.23944      -0.37      0.7246
             E             1       1.71244        0.18747       9.13      <.0001

                        A versus E REGRESSION on TEMPERATURE=35
12
                                                           18:38 Saturday, October
25, 2003

         Obs           Temp              E             A      EbyA       Einv

           1             35          0.001         0.001       .          .
           2             35         0.3619          0.12    3.01583    2.76319
           3             35         0.6896          1.11    0.62126    1.45012
           4             35          0.997          2.01    0.49602    1.00301
           5             35           1.14          1.73    0.65896    0.87719
           6             35          1.556          2.85    0.54596    0.64267
           7             35          1.857          3.38    0.54941    0.53850
           8             35          2.069   2.941111111    0.70348    0.48333

         Obs      Ainv        LNE          LNA      CMBLNGE      PRED        RESID

           1       .           .            .          .        -0.08670     0.08770
           2    8.33333    -1.01639    -2.12026    1.66229     0.53132    -0.41132
           3    0.90090    -0.37164     0.10436    1.20421     1.09248     0.01752
           4    0.49751    -0.00300     0.69813    1.00150     1.61889     0.39111
           5    0.57803     0.13103     0.54812    0.93659     1.86377    -0.13377
           6    0.35088     0.44212     1.04732    0.80167     2.57614     0.27386
           7    0.29586     0.61896     1.21788    0.73383     3.09159     0.28841
```

```
            8     0.34001     0.72707     1.07879    0.69522     3.45462    -0.51351

                           EbyA versus E REGRESSION on TEMPERATURE=35
13
                                                                18:38 Saturday, October
25, 2003

                                       The REG Procedure
                                         Model: MODEL1
                                   Dependent Variable: EbyA

                                      Analysis of Variance

                                             Sum of           Mean
         Source                   DF        Squares         Square    F Value    Pr > F

         Model                     1        1.85954        1.85954       2.91    0.1485
         Error                     5        3.19099        0.63820
         Corrected Total           6        5.05053


                      Root MSE              0.79887    R-Square     0.3682
                      Dependent Mean        0.94156    Adj R-Sq     0.2418
                      Coeff Var            84.84563


                                      Parameter Estimates

                                   Parameter       Standard
              Variable     DF       Estimate          Error    t Value    Pr > |t|

              Intercept     1        2.05269        0.71756       2.86      0.0354
              E             1       -0.89706        0.52553      -1.71      0.1485

                           EbyA versus E REGRESSION on TEMPERATURE=35
14
                                                                18:38 Saturday, October
25, 2003

           Obs           Temp              E               A      EbyA       Einv

            1              35          0.001           0.001      .          .
            2              35         0.3619            0.12    3.01583    2.76319
            3              35         0.6896            1.11    0.62126    1.45012
            4              35          0.997            2.01    0.49602    1.00301
            5              35           1.14            1.73    0.65896    0.87719
            6              35          1.556            2.85    0.54596    0.64267
            7              35          1.857            3.38    0.54941    0.53850
            8              35          2.069     2.941111111    0.70348    0.48333

           Obs      Ainv        LNE         LNA      CMBLNGE      PRED       RESID

            1     .             .           .          .        2.05180       .
            2     8.33333    -1.01639    -2.12026    1.66229    1.72805     1.28778
            3     0.90090    -0.37164     0.10436    1.20421    1.43408    -0.81282
            4     0.49751    -0.00300     0.69813    1.00150    1.15833    -0.66231
            5     0.57803     0.13103     0.54812    0.93659    1.03005    -0.37109
            6     0.35088     0.44212     1.04732    0.80167    0.65687    -0.11091
            7     0.29586     0.61896     1.21788    0.73383    0.38686     0.16255
            8     0.34001     0.72707     1.07879    0.69522    0.19668     0.50679

                             LANGMUIR REGRESSION on TEMPERATURE=35
15
                                                                18:38 Saturday, October
25, 2003

                                       The REG Procedure
                                         Model: MODEL1
                                   Dependent Variable: Ainv

                                      Analysis of Variance
```

```
                                             Sum of           Mean
         Source                   DF        Squares         Square    F Value    Pr > F

         Model                     1       46.39448       46.39448      35.47    0.0019
         Error                     5        6.53927        1.30785
         Corrected Total           6       52.93375


                      Root MSE              1.14361    R-Square     0.8765
                      Dependent Mean        1.61379    Adj R-Sq     0.8518
                      Coeff Var            70.86518


                                      Parameter Estimates

                                   Parameter       Standard
              Variable     DF       Estimate          Error    t Value    Pr > |t|

              Intercept     1       -2.23247        0.77709      -2.87      0.0349
              Einv          1        3.47045        0.58268       5.96      0.0019

                             LANGMUIR REGRESSION on TEMPERATURE=35
16
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           Obs           Temp              E               A      EbyA       Einv

            1              35          0.001           0.001     .          .
            2              35         0.3619            0.12    3.01583    2.76319
            3              35         0.6896            1.11    0.62126    1.45012
            4              35          0.997            2.01    0.49602    1.00301
            5              35           1.14            1.73    0.65896    0.87719
            6              35          1.556            2.85    0.54596    0.64267
            7              35          1.857            3.38    0.54941    0.53850
            8              35          2.069     2.941111111    0.70348    0.48333

           Obs      Ainv        LNE         LNA      CMBLNGE      PRED        RESID

            1      .           .           .          .           .           .
            2     8.33333    -1.01639    -2.12026    1.66229     7.35706     0.97628
            3     0.90090    -0.37164     0.10436    1.20421     2.80009    -1.89919
            4     0.49751    -0.00300     0.69813    1.00150     1.24842    -0.75091
            5     0.57803     0.13103     0.54812    0.93659     0.81179    -0.23375
            6     0.35088     0.44212     1.04732    0.80167    -0.00210     0.35298
            7     0.29586     0.61896     1.21788    0.73383    -0.36362     0.65948
            8     0.34001     0.72707     1.07879    0.69522    -0.55511     0.89512

                            FREUNDLICH REGRESSION on TEMPERATURE=35
17
                                                                18:38 Saturday, October
25, 2003

                                       The REG Procedure
                                         Model: MODEL1
                                    Dependent Variable: LNA

                                      Analysis of Variance

                                             Sum of           Mean
         Source                   DF        Squares         Square    F Value    Pr > F

         Model                     1        7.06807        7.06807      34.54    0.0020
         Error                     5        1.02331        0.20466
         Corrected Total           6        8.09138


                      Root MSE              0.45239    R-Square     0.8735
                      Dependent Mean        0.36776    Adj R-Sq     0.8482
                      Coeff Var           123.01293
```

```
                                      Parameter Estimates

                                   Parameter       Standard
              Variable     DF       Estimate          Error    t Value    Pr > |t|

              Intercept     1        0.23421        0.17249       1.36      0.2326
              LNE           1        1.77015        0.30122       5.88      0.0020

                            FREUNDLICH REGRESSION on TEMPERATURE=35
18
                                                                 18:38 Saturday, October
25, 2003

           Obs            Temp                E               A      EbyA       Einv

            1               35            0.001           0.001     .          .
            2               35           0.3619            0.12    3.01583    2.76319
            3               35           0.6896            1.11    0.62126    1.45012
            4               35            0.997            2.01    0.49602    1.00301
            5               35             1.14            1.73    0.65896    0.87719
            6               35            1.556            2.85    0.54596    0.64267
            7               35            1.857            3.38    0.54941    0.53850
            8               35            2.069     2.941111111    0.70348    0.48333

           Obs      Ainv        LNE         LNA      CMBLNGE      PRED        RESID

            1       .           .           .          .           .           .
            2     8.33333    -1.01639    -2.12026    1.66229    -1.56495    -0.55531
            3     0.90090    -0.37164     0.10436    1.20421    -0.42366     0.52802
            4     0.49751    -0.00300     0.69813    1.00150     0.22889     0.46925
            5     0.57803     0.13103     0.54812    0.93659     0.46615     0.08197
            6     0.35088     0.44212     1.04732    0.80167     1.01682     0.03050
            7     0.29586     0.61896     1.21788    0.73383     1.32986    -0.11199
            8     0.34001     0.72707     1.07879    0.69522     1.52122    -0.44243

                         COMBINED LANGMUIR REGRESSION on TEMPERATURE=35
19
                                                                 18:38 Saturday, October
25, 2003

                                       The REG Procedure
                                         Model: MODEL1
                                   Dependent Variable: Ainv

                                      Analysis of Variance

                                             Sum of           Mean
         Source                   DF        Squares         Square    F Value    Pr > F

         Model                     1       41.77570       41.77570      18.72    0.0075
         Error                     5       11.15806        2.23161
         Corrected Total           6       52.93375


                      Root MSE               1.49386    R-Square     0.7892
                      Dependent Mean         1.61379    Adj R-Sq     0.7470
                      Coeff Var             92.56834


                                      Parameter Estimates

                                   Parameter       Standard
              Variable     DF       Estimate          Error    t Value    Pr > |t|

              Intercept     1       -6.22214        1.89705      -3.28      0.0220
              CMBLNGE       1        7.79661        1.80199       4.33      0.0075

                         COMBINED LANGMUIR REGRESSION on TEMPERATURE=35
20
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                                                        18:38 Saturday, October
25, 2003

         Obs           Temp              E               A      EbyA       Einv

           1             35          0.001           0.001     .          .
           2             35         0.3619            0.12    3.01583    2.76319
           3             35         0.6896            1.11    0.62126    1.45012
           4             35          0.997            2.01    0.49602    1.00301
           5             35           1.14            1.73    0.65896    0.87719
           6             35          1.556            2.85    0.54596    0.64267
           7             35          1.857            3.38    0.54941    0.53850
           8             35          2.069     2.941111111    0.70348    0.48333

         Obs      Ainv        LNE         LNA      CMBLNGE      PRED        RESID

           1      .          .           .          .          .           .
           2     8.33333   -1.01639    -2.12026    1.66229    6.73806     1.59527
           3     0.90090   -0.37164     0.10436    1.20421    3.16660    -2.26570
           4     0.49751   -0.00300     0.69813    1.00150    1.58620    -1.08869
           5     0.57803    0.13103     0.54812    0.93659    1.08006    -0.50203
           6     0.35088    0.44212     1.04732    0.80167    0.02817     0.32271
           7     0.29586    0.61896     1.21788    0.73383   -0.50077     0.79662
           8     0.34001    0.72707     1.07879    0.69522   -0.80181     1.14181

                        A versus E REGRESSION on TEMPERATURE=45
21
                                                        18:38 Saturday, October
25, 2003

                                   The REG Procedure
                                    Model: MODEL1
                                 Dependent Variable: A

                                  Analysis of Variance

                                         Sum of           Mean
       Source                   DF        Squares         Square    F Value    Pr > F

       Model                     1       17.61561       17.61561     210.08    <.0001
       Error                     6        0.50311        0.08385
       Corrected Total           7       18.11872


                    Root MSE              0.28957    R-Square     0.9722
                    Dependent Mean        2.07249    Adj R-Sq     0.9676
                    Coeff Var            13.97223


                                  Parameter Estimates

                                 Parameter       Standard
            Variable     DF       Estimate          Error    t Value    Pr > |t|

            Intercept     1       -0.30841        0.19356      -1.59      0.1622
            E             1        2.19653        0.15155      14.49      <.0001

                        A versus E REGRESSION on TEMPERATURE=45
22
                                                        18:38 Saturday, October
25, 2003

         Obs           Temp              E               A      EbyA       Einv

           1             45          0.001           0.001     .          .
           2             45         0.3619             0.2    1.80950    2.76319
           3             45         0.6896            1.17    0.58940    1.45012
           4             45          0.997            1.92    0.51927    1.00301
           5             45           1.14            1.89    0.60317    0.87719
           6             45          1.556            3.23    0.48173    0.64267
           7             45          1.857            4.17    0.44532    0.53850
```

```
            8               45           2.069     3.998888889    0.51739    0.48333

           Obs      Ainv        LNE         LNA      CMBLNGE      PRED        RESID

            1      .           .           .           .        -0.30621     0.30721
            2     5.00000    -1.01639    -1.60944    1.66229     0.48651    -0.28651
            3     0.85470    -0.37164     0.15700    1.20421     1.20631    -0.03631
            4     0.52083    -0.00300     0.65233    1.00150     1.88153     0.03847
            5     0.52910     0.13103     0.63658    0.93659     2.19563    -0.30563
            6     0.30960     0.44212     1.17248    0.80167     3.10938     0.12062
            7     0.23981     0.61896     1.42792    0.73383     3.77054     0.39946
            8     0.25007     0.72707     1.38602    0.69522     4.23620    -0.23731

                           EbyA versus E REGRESSION on TEMPERATURE=45
23
                                                                18:38 Saturday, October
25, 2003

                                       The REG Procedure
                                         Model: MODEL1
                                   Dependent Variable: EbyA

                                      Analysis of Variance

                                             Sum of           Mean
         Source                   DF        Squares         Square    F Value    Pr > F

         Model                     1        0.66237        0.66237       4.31    0.0925
         Error                     5        0.76812        0.15362
         Corrected Total           6        1.43049


                      Root MSE              0.39195    R-Square     0.4630
                      Dependent Mean        0.70940    Adj R-Sq     0.3556
                      Coeff Var            55.25085


                                      Parameter Estimates

                                   Parameter       Standard
              Variable     DF       Estimate          Error    t Value    Pr > |t|

              Intercept     1        1.37255        0.35206       3.90      0.0114
              E             1       -0.53538        0.25784      -2.08      0.0925

                           EbyA versus E REGRESSION on TEMPERATURE=45
24
                                                                18:38 Saturday, October
25, 2003

           Obs            Temp               E               A      EbyA       Einv

            1               45           0.001           0.001      .          .
            2               45          0.3619             0.2    1.80950    2.76319
            3               45          0.6896            1.17    0.58940    1.45012
            4               45           0.997            1.92    0.51927    1.00301
            5               45            1.14            1.89    0.60317    0.87719
            6               45           1.556            3.23    0.48173    0.64267
            7               45           1.857            4.17    0.44532    0.53850
            8               45           2.069     3.998888889    0.51739    0.48333

           Obs      Ainv        LNE         LNA      CMBLNGE      PRED       RESID

            1      .           .           .           .        1.37201      .
            2     5.00000    -1.01639    -1.60944    1.66229    1.17879     0.63071
            3     0.85470    -0.37164     0.15700    1.20421    1.00335    -0.41395
            4     0.52083    -0.00300     0.65233    1.00150    0.83877    -0.31950
            5     0.52910     0.13103     0.63658    0.93659    0.76221    -0.15904
            6     0.30960     0.44212     1.17248    0.80167    0.53949    -0.05776
            7     0.23981     0.61896     1.42792    0.73383    0.37834     0.06698
            8     0.25007     0.72707     1.38602    0.69522    0.26484     0.25255
```

```
                              LANGMUIR REGRESSION on TEMPERATURE=45
25
                                                                  18:38 Saturday, October
25, 2003

                                        The REG Procedure
                                          Model: MODEL1
                                     Dependent Variable: Ainv

                                       Analysis of Variance

                                                Sum of           Mean
         Source                   DF           Squares         Square    F Value    Pr > F

         Model                     1          16.41774       16.41774      51.28    0.0008
         Error                     5           1.60084        0.32017
         Corrected Total           6          18.01858


                      Root MSE              0.56583    R-Square     0.9112
                      Dependent Mean        1.10059    Adj R-Sq     0.8934
                      Coeff Var            51.41192


                                       Parameter Estimates

                                    Parameter       Standard
              Variable     DF        Estimate          Error    t Value    Pr > |t|

              Intercept     1        -1.18744        0.38449      -3.09      0.0272
              Einv          1         2.06447        0.28830       7.16      0.0008

                              LANGMUIR REGRESSION on TEMPERATURE=45
26
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           Obs          Temp              E              A      EbyA       Einv

            1             45          0.001          0.001       .          .
            2             45         0.3619            0.2    1.80950    2.76319
            3             45         0.6896           1.17    0.58940    1.45012
            4             45          0.997           1.92    0.51927    1.00301
            5             45           1.14           1.89    0.60317    0.87719
            6             45          1.556           3.23    0.48173    0.64267
            7             45          1.857           4.17    0.44532    0.53850
            8             45          2.069    3.998888889    0.51739    0.48333

           Obs      Ainv         LNE         LNA      CMBLNGE       PRED        RESID

            1        .           .           .           .           .           .
            2     5.00000    -1.01639    -1.60944    1.66229     4.51710     0.48290
            3     0.85470    -0.37164     0.15700    1.20421     1.80628    -0.95158
            4     0.52083    -0.00300     0.65233    1.00150     0.88324    -0.36241
            5     0.52910     0.13103     0.63658    0.93659     0.62350    -0.09440
            6     0.30960     0.44212     1.17248    0.80167     0.13934     0.17026
            7     0.23981     0.61896     1.42792    0.73383    -0.07572     0.31553
            8     0.25007     0.72707     1.38602    0.69522    -0.18963     0.43970

                             FREUNDLICH REGRESSION on TEMPERATURE=45
27
                                                                  18:38 Saturday, October
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                                        The REG Procedure
                                          Model: MODEL1
                                     Dependent Variable: LNA

                                       Analysis of Variance
```

```
                                             Sum of           Mean
         Source                   DF        Squares         Square    F Value    Pr > F

         Model                     1        6.33572        6.33572      88.76    0.0002
         Error                     5        0.35690        0.07138
         Corrected Total           6        6.69262


                      Root MSE              0.26717    R-Square     0.9467
                      Dependent Mean        0.54613    Adj R-Sq     0.9360
                      Coeff Var            48.92089


                                      Parameter Estimates

                                   Parameter       Standard
              Variable     DF       Estimate          Error    t Value    Pr > |t|

              Intercept     1        0.41968        0.10187       4.12      0.0092
              LNE           1        1.67594        0.17789       9.42      0.0002

                            FREUNDLICH REGRESSION on TEMPERATURE=45
28
                                                                18:38 Saturday, October
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           Obs            Temp               E               A      EbyA       Einv

            1               45           0.001           0.001     .           .
            2               45          0.3619             0.2    1.80950    2.76319
            3               45          0.6896            1.17    0.58940    1.45012
            4               45           0.997            1.92    0.51927    1.00301
            5               45            1.14            1.89    0.60317    0.87719
            6               45           1.556            3.23    0.48173    0.64267
            7               45           1.857            4.17    0.44532    0.53850
            8               45           2.069     3.998888889    0.51739    0.48333

           Obs      Ainv        LNE          LNA      CMBLNGE      PRED        RESID

            1      .           .           .           .          .            .
            2     5.00000    -1.01639    -1.60944    1.66229    -1.28372    -0.32572
            3     0.85470    -0.37164     0.15700    1.20421    -0.20317     0.36018
            4     0.52083    -0.00300     0.65233    1.00150     0.41464     0.23768
            5     0.52910     0.13103     0.63658    0.93659     0.63927    -0.00270
            6     0.30960     0.44212     1.17248    0.80167     1.16064     0.01184
            7     0.23981     0.61896     1.42792    0.73383     1.45702    -0.02910
            8     0.25007     0.72707     1.38602    0.69522     1.63819    -0.25218

                         COMBINED LANGMUIR REGRESSION on TEMPERATURE=45
29
                                                                18:38 Saturday, October
25, 2003

                                       The REG Procedure
                                         Model: MODEL1
                                   Dependent Variable: Ainv

                                      Analysis of Variance

                                             Sum of           Mean
         Source                   DF        Squares         Square    F Value    Pr > F

         Model                     1       15.02045       15.02045      25.05    0.0041
         Error                     5        2.99813        0.59963
         Corrected Total           6       18.01858


                      Root MSE              0.77436    R-Square     0.8336
                      Dependent Mean        1.10059    Adj R-Sq     0.8003
                      Coeff Var            70.35839
```

```
                                     Parameter Estimates

                                  Parameter       Standard
              Variable     DF      Estimate          Error    t Value    Pr > |t|

              Intercept     1      -3.59803        0.98336      -3.66      0.0146
              CMBLNGE       1       4.67504        0.93408       5.00      0.0041

                          COMBINED LANGMUIR REGRESSION on TEMPERATURE=45
30
                                                                 18:38 Saturday, October
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            Obs           Temp              E               A      EbyA       Einv

              1             45          0.001           0.001       .          .
              2             45         0.3619             0.2    1.80950    2.76319
              3             45         0.6896            1.17    0.58940    1.45012
              4             45          0.997            1.92    0.51927    1.00301
              5             45           1.14            1.89    0.60317    0.87719
              6             45          1.556            3.23    0.48173    0.64267
              7             45          1.857            4.17    0.44532    0.53850
              8             45          2.069     3.998888889    0.51739    0.48333

            Obs      Ainv        LNE         LNA      CMBLNGE      PRED        RESID

              1        .           .           .          .          .           .
              2     5.00000    -1.01639    -1.60944    1.66229     4.17323     0.82677
              3     0.85470    -0.37164     0.15700    1.20421     2.03169    -1.17699
              4     0.52083    -0.00300     0.65233    1.00150     1.08404    -0.56321
              5     0.52910     0.13103     0.63658    0.93659     0.78055    -0.25145
              6     0.30960     0.44212     1.17248    0.80167     0.14981     0.15979
              7     0.23981     0.61896     1.42792    0.73383    -0.16735     0.40716
              8     0.25007     0.72707     1.38602    0.69522    -0.34786     0.59793

                          A versus E REGRESSION on TEMPERATURE=55
31
                                                                 18:38 Saturday, October
25, 2003

                                      The REG Procedure
                                        Model: MODEL1
                                    Dependent Variable: A

                                     Analysis of Variance

                                            Sum of           Mean
         Source                   DF       Squares         Square    F Value    Pr > F

         Model                     1      23.69622       23.69622     192.06    <.0001
         Error                     6       0.74028        0.12338
         Corrected Total           7      24.43650


                      Root MSE              0.35125    R-Square     0.9697
                      Dependent Mean        2.24749    Adj R-Sq     0.9647
                      Coeff Var            15.62878


                                     Parameter Estimates

                                  Parameter       Standard
              Variable     DF      Estimate          Error    t Value    Pr > |t|

              Intercept     1      -0.51393        0.23479      -2.19      0.0712
              E             1       2.54757        0.18383      13.86      <.0001

                          A versus E REGRESSION on TEMPERATURE=55
32
                                                                 18:38 Saturday, October
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25, 2003

           Obs            Temp               E               A      EbyA       Einv

            1               55           0.001           0.001     .          .
            2               55          0.3619            0.18    2.01056    2.76319
            3               55          0.6896            0.97    0.71093    1.45012
            4               55           0.997            1.85    0.53892    1.00301
            5               55            1.14            2.04    0.55882    0.87719
            6               55           1.556            3.75    0.41493    0.64267
            7               55           1.857            4.53    0.40993    0.53850
            8               55           2.069     4.658888889    0.44410    0.48333

           Obs      Ainv        LNE         LNA      CMBLNGE      PRED         RESID

            1      .           .           .          .         -0.51138     0.51238
            2     5.55556    -1.01639    -1.71480    1.66229     0.40804    -0.22804
            3     1.03093    -0.37164    -0.03046    1.20421     1.24288    -0.27288
            4     0.54054    -0.00300     0.61519    1.00150     2.02601    -0.17601
            5     0.49020     0.13103     0.71295    0.93659     2.39031    -0.35031
            6     0.26667     0.44212     1.32176    0.80167     3.45010     0.29990
            7     0.22075     0.61896     1.51072    0.73383     4.21692     0.31308
            8     0.21464     0.72707     1.53878    0.69522     4.75701    -0.09812

                           EbyA versus E REGRESSION on TEMPERATURE=55
33
                                                                18:38 Saturday, October
25, 2003

                                       The REG Procedure
                                         Model: MODEL1
                                   Dependent Variable: EbyA

                                      Analysis of Variance

                                             Sum of           Mean
         Source                   DF        Squares         Square    F Value    Pr > F

         Model                     1        1.08646        1.08646       6.02    0.0577
         Error                     5        0.90292        0.18058
         Corrected Total           6        1.98938


                      Root MSE              0.42495    R-Square     0.5461
                      Dependent Mean        0.72688    Adj R-Sq     0.4554
                      Coeff Var            58.46210


                                      Parameter Estimates

                                   Parameter       Standard
              Variable     DF       Estimate          Error    t Value    Pr > |t|

              Intercept     1        1.57620        0.38170       4.13      0.0091
              E             1       -0.68568        0.27955      -2.45      0.0577

                           EbyA versus E REGRESSION on TEMPERATURE=55
34
                                                                18:38 Saturday, October
25, 2003

           Obs            Temp               E               A      EbyA       Einv

            1               55           0.001           0.001     .          .
            2               55          0.3619            0.18    2.01056    2.76319
            3               55          0.6896            0.97    0.71093    1.45012
            4               55           0.997            1.85    0.53892    1.00301
            5               55            1.14            2.04    0.55882    0.87719
            6               55           1.556            3.75    0.41493    0.64267
            7               55           1.857            4.53    0.40993    0.53850
            8               55           2.069     4.658888889    0.44410    0.48333
```

```
          Obs      Ainv        LNE         LNA      CMBLNGE      PRED       RESID

            1        .          .           .          .        1.57552       .
            2     5.55556   -1.01639    -1.71480    1.66229    1.32805     0.68250
            3     1.03093   -0.37164    -0.03046    1.20421    1.10335    -0.39243
            4     0.54054   -0.00300     0.61519    1.00150    0.89257    -0.35366
            5     0.49020    0.13103     0.71295    0.93659    0.79452    -0.23570
            6     0.26667    0.44212     1.32176    0.80167    0.50928    -0.09434
            7     0.22075    0.61896     1.51072    0.73383    0.30289     0.10705
            8     0.21464    0.72707     1.53878    0.69522    0.15752     0.28658

                           LANGMUIR REGRESSION on TEMPERATURE=55
35
                                                           18:38 Saturday, October
25, 2003

                                     The REG Procedure
                                       Model: MODEL1
                                  Dependent Variable: Ainv

                                    Analysis of Variance

                                             Sum of           Mean
         Source                   DF        Squares         Square    F Value    Pr > F

         Model                     1       20.99046       20.99046      60.05    0.0006
         Error                     5        1.74773        0.34955
         Corrected Total           6       22.73820


                      Root MSE              0.59122    R-Square     0.9231
                      Dependent Mean        1.18847    Adj R-Sq     0.9078
                      Coeff Var            49.74679


                                    Parameter Estimates

                                   Parameter       Standard
              Variable     DF       Estimate          Error    t Value    Pr > |t|

              Intercept     1       -1.39865        0.40174      -3.48      0.0176
              Einv          1        2.33434        0.30123       7.75      0.0006

                           LANGMUIR REGRESSION on TEMPERATURE=55
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          Obs           Temp              E              A      EbyA       Einv

            1             55          0.001          0.001       .          .
            2             55         0.3619           0.18    2.01056    2.76319
            3             55         0.6896           0.97    0.71093    1.45012
            4             55          0.997           1.85    0.53892    1.00301
            5             55           1.14           2.04    0.55882    0.87719
            6             55          1.556           3.75    0.41493    0.64267
            7             55          1.857           4.53    0.40993    0.53850
            8             55          2.069    4.658888889    0.44410    0.48333

          Obs      Ainv        LNE         LNA      CMBLNGE      PRED        RESID

            1        .          .           .          .          .           .
            2     5.55556   -1.01639    -1.71480    1.66229     5.05158     0.50398
            3     1.03093   -0.37164    -0.03046    1.20421     1.98641    -0.95548
            4     0.54054   -0.00300     0.61519    1.00150     0.94271    -0.40217
            5     0.49020    0.13103     0.71295    0.93659     0.64902    -0.15882
            6     0.26667    0.44212     1.32176    0.80167     0.10157     0.16510
            7     0.22075    0.61896     1.51072    0.73383    -0.14160     0.36235
            8     0.21464    0.72707     1.53878    0.69522    -0.27040     0.48505
```

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                            FREUNDLICH REGRESSION on TEMPERATURE=55
37
                                                                18:38 Saturday, October
25, 2003

                                       The REG Procedure
                                         Model: MODEL1
                                    Dependent Variable: LNA

                                      Analysis of Variance

                                             Sum of           Mean
         Source                   DF        Squares         Square    F Value    Pr > F

         Model                     1        7.76702        7.76702     172.79    <.0001
         Error                     5        0.22475        0.04495
         Corrected Total           6        7.99177


                      Root MSE              0.21201    R-Square     0.9719
                      Dependent Mean        0.56488    Adj R-Sq     0.9663
                      Coeff Var            37.53259


                                      Parameter Estimates

                                   Parameter       Standard
              Variable     DF       Estimate          Error    t Value    Pr > |t|

              Intercept     1        0.42487        0.08084       5.26      0.0033
              LNE           1        1.85561        0.14116      13.15      <.0001
                            FREUNDLICH REGRESSION on TEMPERATURE=55
38
                                                                18:38 Saturday, October
25, 2003

           Obs           Temp               E               A      EbyA       Einv

            1              55           0.001           0.001     .          .
            2              55          0.3619            0.18    2.01056    2.76319
            3              55          0.6896            0.97    0.71093    1.45012
            4              55           0.997            1.85    0.53892    1.00301
            5              55            1.14            2.04    0.55882    0.87719
            6              55           1.556            3.75    0.41493    0.64267
            7              55           1.857            4.53    0.40993    0.53850
            8              55           2.069     4.658888889    0.44410    0.48333

           Obs      Ainv        LNE         LNA      CMBLNGE       PRED        RESID

            1      .           .           .          .           .           .
            2     5.55556    -1.01639    -1.71480    1.66229    -1.46115    -0.25365
            3     1.03093    -0.37164    -0.03046    1.20421    -0.26475     0.23429
            4     0.54054    -0.00300     0.61519    1.00150     0.41930     0.19589
            5     0.49020     0.13103     0.71295    0.93659     0.66801     0.04494
            6     0.26667     0.44212     1.32176    0.80167     1.24527     0.07648
            7     0.22075     0.61896     1.51072    0.73383     1.57343    -0.06270
            8     0.21464     0.72707     1.53878    0.69522     1.77402    -0.23525
                         COMBINED LANGMUIR REGRESSION on TEMPERATURE=55
39
                                                                18:38 Saturday, October
25, 2003

                                       The REG Procedure
                                         Model: MODEL1
                                   Dependent Variable: Ainv

                                      Analysis of Variance

                                             Sum of           Mean
```

```
         Source                   DF       Squares         Square    F Value    Pr > F

         Model                     1      19.28568       19.28568      27.93    0.0032
         Error                     5       3.45251        0.69050
         Corrected Total           6      22.73820


                      Root MSE               0.83096   R-Square     0.8482
                      Dependent Mean         1.18847   Adj R-Sq     0.8178
                      Coeff Var             69.91895


                                      Parameter Estimates

                                   Parameter       Standard
              Variable     DF       Estimate          Error    t Value    Pr > |t|

              Intercept     1       -4.13563        1.05524      -3.92      0.0112
              CMBLNGE       1        5.29739        1.00237       5.28      0.0032

                         COMBINED LANGMUIR REGRESSION on TEMPERATURE=55
40
                                                                18:38 Saturday, October
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           Obs             Temp               E               A      EbyA      Einv

            1                55           0.001           0.001    .          .
            2                55          0.3619            0.18    2.01056    2.76319
            3                55          0.6896            0.97    0.71093    1.45012
            4                55           0.997            1.85    0.53892    1.00301
            5                55            1.14            2.04    0.55882    0.87719
            6                55           1.556            3.75    0.41493    0.64267
            7                55           1.857            4.53    0.40993    0.53850
            8                55           2.069     4.658888889    0.44410    0.48333

           Obs        Ainv       LNE         LNA      CMBLNGE      PRED        RESID

            1      .           .           .          .           .           .
            2      5.55556    -1.01639    -1.71480    1.66229     4.67014     0.88541
            3      1.03093    -0.37164    -0.03046    1.20421     2.24352    -1.21260
            4      0.54054    -0.00300     0.61519    1.00150     1.16972    -0.62918
            5      0.49020     0.13103     0.71295    0.93659     0.82583    -0.33563
            6      0.26667     0.44212     1.32176    0.80167     0.11112     0.15554
            7      0.22075     0.61896     1.51072    0.73383    -0.24826     0.46901
            8      0.21464     0.72707     1.53878    0.69522    -0.45280     0.66744
```

Appendix D

Statistical Analysis for Average values of Experimental Adsorption Data of CBH I, CBH II and EG II to fit Adsorption Models

SAS CODE

```
%let path=d:\Dole Files\;
libname bus "&path.";
/* THIS SAS FILE IS FOR REGRESSION FOR DATA ON AVERAGE FILE */
/* THIS DATASET CONTAINS AVERAGE OF ALL THREE FILES CBHIa CBHIIa and
EGIIa */

/* This step makes new variables for calculating different models */

data temp;
set bus.average;
m=2;
EbyA=E/A;
Einv=1/E;
Ainv=1/A;
LNE=log(E);
LNA=log(A);
CMBLNGE=1/(E**(1/m));
drop m;
run;

/*********************************************/
/* regression model...For AVERAGE OF FILE CBHI */
/*********************************************/

/* Selecting Cases */

data tempCBHI;
set temp;
if label='CBHI' then output;
run;


/* A versus E Regression */
title 'A versus E REGRESSION on CBHI AVERAGES';
PROC REG data=tempCBHI;
  MODEL A=E;
OUTPUT OUT=AE P=PRED R=RESID;
PLOT A*E='*' PREDICTED.*E='o' RESIDUAL.*E='x';
PROC PRINT DATA=AE;
run;


/* EbyA versus E Regression */
title 'EbyA versus E REGRESSION on CBHI AVERAGES';
PROC REG data=tempCBHI;
  MODEL EbyA=E;
OUTPUT OUT=EbyA P=PRED R=RESID;
PLOT EbyA*E='*' PREDICTED.*E='o' RESIDUAL.*E='x';
PROC PRINT DATA=EbyA;
run;


/* Langmuir Regression */
title 'LANGMUIR REGRESSION on CBHI AVERAGES';
PROC REG data=tempCBHI;
```

```
  MODEL Ainv=Einv;
OUTPUT OUT=LANGMUIR P=PRED R=RESID;
PLOT Ainv*Einv='*' PREDICTED.*Einv='o' RESIDUAL.*Einv='x';
PROC PRINT DATA=LANGMUIR;
run;


/* Freundlich Regression */
title 'FREUNDLICH REGRESSION on CBHI AVERAGES';
PROC REG data=tempCBHI;
MODEL LNA=LNE;
OUTPUT OUT=FREUNDLICH P=PRED R=RESID;
PLOT LNA*LNE='*' PREDICTED.*LNE='o' RESIDUAL.*LNE='x';
PROC PRINT DATA=FREUNDLICH;
run;


/* Combined Langmuir Regression */
title 'COMBINED LANGMUIR REGRESSION on CBHI AVERAGES';
PROC REG data=tempCBHI;
MODEL Ainv=CMBLNGE;
OUTPUT OUT=COMBLANGMUIR P=PRED R=RESID;
PLOT Ainv*CMBLNGE='*' PREDICTED.*CMBLNGE='o' RESIDUAL.*CMBLNGE='x';
PROC PRINT DATA=COMBLANGMUIR;
run;



/**************************************************/
/* regression model....FOR AVERAGES OF FILE CBHII */
/**************************************************/

/* Selecting Cases */

data tempCBHII;
set temp;
if label='CBHII' then output;
run;

/* A versus E Regression */
title 'A versus E REGRESSION on AVERAGES OF FILE CBHII';
PROC REG data=tempCBHII;
  MODEL A=E;
OUTPUT OUT=AE P=PRED R=RESID;
PLOT A*E='*' PREDICTED.*E='o' RESIDUAL.*E='x';
PROC PRINT DATA=AE;
run;


/* EbyA versus E Regression */
title 'EbyA versus E REGRESSION on AVERAGES OF FILE CBHII';
PROC REG data=tempCBHII;
  MODEL EbyA=E;
OUTPUT OUT=EbyA P=PRED R=RESID;
PLOT EbyA*E='*' PREDICTED.*E='o' RESIDUAL.*E='x';
PROC PRINT DATA=EbyA;
run;
```

```
/* Langmuir Regression */
title 'LANGMUIR REGRESSION on AVERAGES OF FILE CBHII';
PROC REG data=tempCBHII;
  MODEL Ainv=Einv;
OUTPUT OUT=LANGMUIR P=PRED R=RESID;
PLOT Ainv*Einv='*' PREDICTED.*Einv='o' RESIDUAL.*Einv='x';
PROC PRINT DATA=LANGMUIR;
run;

/* Freundlich Regression */
title 'FREUNDLICH REGRESSION on AVERAGES OF FILE CBHII';
PROC REG data=tempCBHII;
MODEL LNA=LNE;
OUTPUT OUT=FREUNDLICH P=PRED R=RESID;
PLOT LNA*LNE='*' PREDICTED.*LNE='o' RESIDUAL.*LNE='x';
PROC PRINT DATA=FREUNDLICH;
run;

/* Combined Langmuir Regression */
title 'COMBINED LANGMUIR REGRESSION on AVERAGES OF FILE CBHII';
PROC REG data=tempCBHII;
MODEL Ainv=CMBLNGE;
OUTPUT OUT=COMBLANGMUIR P=PRED R=RESID;
PLOT Ainv*CMBLNGE='*' PREDICTED.*CMBLNGE='o' RESIDUAL.*CMBLNGE='x';
PROC PRINT DATA=COMBLANGMUIR;
run;



/**************************************************/
/* regression model....FOR AVERAGES OF FILE EGII */
/**************************************************/

/* Selecting Cases */

data tempEGII;
set temp;
if label='EGII' then output;
run;

/* A versus E Regression */
title 'A versus E REGRESSION on AVERAGES OF FILE EGII';
PROC REG data=tempEGII;
  MODEL A=E;
OUTPUT OUT=AE P=PRED R=RESID;
PLOT A*E='*' PREDICTED.*E='o' RESIDUAL.*E='x';
PROC PRINT DATA=AE;
run;


/* EbyA versus E Regression */
title 'EbyA versus E REGRESSION on AVERAGES OF FILE EGII';
PROC REG data=tempEGII;
  MODEL EbyA=E;
OUTPUT OUT=EbyA P=PRED R=RESID;
```

```
PLOT EbyA*E='*' PREDICTED.*E='o' RESIDUAL.*E='x';
PROC PRINT DATA=EbyA;
run;


/* Langmuir Regression */
title 'LANGMUIR REGRESSION on AVERAGES OF FILE EGII';
PROC REG data=tempEGII;
  MODEL Ainv=Einv;
OUTPUT OUT=LANGMUIR P=PRED R=RESID;
PLOT Ainv*Einv='*' PREDICTED.*Einv='o' RESIDUAL.*Einv='x';
PROC PRINT DATA=LANGMUIR;
run;

/* Freundlich Regression */
title 'FREUNDLICH REGRESSION on AVERAGES OF FILE EGII';
PROC REG data=tempEGII;
MODEL LNA=LNE;
OUTPUT OUT=FREUNDLICH P=PRED R=RESID;
PLOT LNA*LNE='*' PREDICTED.*LNE='o' RESIDUAL.*LNE='x';
PROC PRINT DATA=FREUNDLICH;
run;

/* Combined Langmuir Regression */
title 'COMBINED LANGMUIR REGRESSION on AVERAGES OF FILE EGII';
PROC REG data=tempEGII;
MODEL Ainv=CMBLNGE;
OUTPUT OUT=COMBLANGMUIR P=PRED R=RESID;
PLOT Ainv*CMBLNGE='*' PREDICTED.*CMBLNGE='o' RESIDUAL.*CMBLNGE='x';
PROC PRINT DATA=COMBLANGMUIR;
run;
```

SAS OUTPUT

```
                          A versus E REGRESSION on CBHI AVERAGES
1
                                                                18:14 Saturday, October
25, 2003

                                       The REG Procedure
                                         Model: MODEL1
                                     Dependent Variable: A

                                      Analysis of Variance

                                             Sum of           Mean
         Source                   DF        Squares         Square    F Value    Pr > F

         Model                     1        5.69690        5.69690     330.67    <.0001
         Error                     5        0.08614        0.01723
         Corrected Total           6        5.78304


                      Root MSE              0.13126    R-Square     0.9851
                      Dependent Mean        1.65143    Adj R-Sq     0.9821
                      Coeff Var             7.94806


                                      Parameter Estimates

                                   Parameter       Standard
              Variable     DF       Estimate          Error    t Value    Pr > |t|

              Intercept     1       -0.21241        0.11387      -1.87      0.1211
              E             1        1.43752        0.07905      18.18      <.0001
                             A versus E REGRESSION on CBHI AVERAGES
2
                                                                18:14 Saturday, October
25, 2003

         Obs    Label               E               A      EbyA       Einv       Ainv

          1     CBHI            0.493           0.515    0.95728    2.02840    1.94175
          2     CBHI            0.737           0.703    1.04836    1.35685    1.42248
          3     CBHI            0.976           1.168    0.83562    1.02459    0.85616
          4     CBHI            1.159            1.45    0.79931    0.86281    0.68966
          5     CBHI            1.412           2.057    0.68644    0.70822    0.48614
          6     CBHI            1.827            2.41    0.75809    0.54735    0.41494
          7     CBHI            2.472           3.257    0.75898    0.40453    0.30703

         Obs       LNE         LNA      CMBLNGE      PRED       RESID

          1     -0.70725    -0.66359    1.42422    0.49628     0.01872
          2     -0.30517    -0.35240    1.16484    0.84704    -0.14404
          3     -0.02429     0.15529    1.01222    1.19060    -0.02260
          4      0.14756     0.37156    0.92888    1.45367    -0.00367
          5      0.34501     0.72125    0.84156    1.81736     0.23964
          6      0.60268     0.87963    0.73983    2.41393    -0.00393
          7      0.90503     1.18081    0.63603    3.34113    -0.08413
                           EbyA versus E REGRESSION on CBHI AVERAGES
3
                                                                18:14 Saturday, October
25, 2003

                                       The REG Procedure
                                         Model: MODEL1
                                   Dependent Variable: EbyA
```

```
                              Analysis of Variance

                                     Sum of           Mean
Source                   DF         Squares         Square    F Value    Pr > F

Model                     1         0.04707        0.04707       4.86    0.0787
Error                     5         0.04844        0.00969
Corrected Total           6         0.09552


             Root MSE              0.09843    R-Square     0.4928
             Dependent Mean        0.83487    Adj R-Sq     0.3914
             Coeff Var            11.78996


                              Parameter Estimates

                             Parameter       Standard
     Variable     DF          Estimate          Error    t Value    Pr > |t|

     Intercept     1           1.00429        0.08539      11.76      <.0001
     E             1          -0.13067        0.05928      -2.20      0.0787

                    EbyA versus E REGRESSION on CBHI AVERAGES
4
                                                       18:14 Saturday, October
25, 2003

         Obs    Label                E               A      EbyA       Einv       Ainv

          1     CBHI             0.493           0.515    0.95728    2.02840    1.94175
          2     CBHI             0.737           0.703    1.04836    1.35685    1.42248
          3     CBHI             0.976           1.168    0.83562    1.02459    0.85616
          4     CBHI             1.159            1.45    0.79931    0.86281    0.68966
          5     CBHI             1.412           2.057    0.68644    0.70822    0.48614
          6     CBHI             1.827            2.41    0.75809    0.54735    0.41494
          7     CBHI             2.472           3.257    0.75898    0.40453    0.30703

         Obs       LNE         LNA      CMBLNGE      PRED       RESID

          1     -0.70725    -0.66359    1.42422    0.93987     0.01741
          2     -0.30517    -0.35240    1.16484    0.90799     0.14038
          3     -0.02429     0.15529    1.01222    0.87676    -0.04114
          4      0.14756     0.37156    0.92888    0.85285    -0.05353
          5      0.34501     0.72125    0.84156    0.81979    -0.13335
          6      0.60268     0.87963    0.73983    0.76556    -0.00747
          7      0.90503     1.18081    0.63603    0.68127     0.07771
                         LANGMUIR REGRESSION on CBHI AVERAGES
5
                                                       18:14 Saturday, October
25, 2003

                               The REG Procedure
                                Model: MODEL1
                            Dependent Variable: Ainv

                              Analysis of Variance

                                     Sum of           Mean
Source                   DF         Squares         Square    F Value    Pr > F

Model                     1         2.11510        2.11510     247.45    <.0001
Error                     5         0.04274        0.00855
Corrected Total           6         2.15783


             Root MSE              0.09245    R-Square     0.9802
             Dependent Mean        0.87402    Adj R-Sq     0.9762
             Coeff Var            10.57794
```

```
                                Parameter Estimates

                                Parameter       Standard
        Variable     DF          Estimate          Error    t Value    Pr > |t|

        Intercept     1          -0.18543        0.07588      -2.44      0.0584
        Einv          1           1.06973        0.06800      15.73      <.0001

                        LANGMUIR REGRESSION on CBHI AVERAGES
6
                                                          18:14 Saturday, October
25, 2003

   Obs    Label              E              A      EbyA       Einv       Ainv

    1     CBHI           0.493          0.515    0.95728    2.02840    1.94175
    2     CBHI           0.737          0.703    1.04836    1.35685    1.42248
    3     CBHI           0.976          1.168    0.83562    1.02459    0.85616
    4     CBHI           1.159           1.45    0.79931    0.86281    0.68966
    5     CBHI           1.412          2.057    0.68644    0.70822    0.48614
    6     CBHI           1.827           2.41    0.75809    0.54735    0.41494
    7     CBHI           2.472          3.257    0.75898    0.40453    0.30703

   Obs       LNE         LNA      CMBLNGE      PRED       RESID

    1     -0.70725    -0.66359    1.42422    1.98441    -0.04266
    2     -0.30517    -0.35240    1.16484    1.26604     0.15644
    3     -0.02429     0.15529    1.01222    0.91061    -0.05444
    4      0.14756     0.37156    0.92888    0.73755    -0.04789
    5      0.34501     0.72125    0.84156    0.57217    -0.08602
    6      0.60268     0.87963    0.73983    0.40008     0.01486
    7      0.90503     1.18081    0.63603    0.24731     0.05972

                       FREUNDLICH REGRESSION on CBHI AVERAGES
7
                                                          18:14 Saturday, October
25, 2003

                                 The REG Procedure
                                   Model: MODEL1
                              Dependent Variable: LNA

                                Analysis of Variance

                                         Sum of           Mean
   Source                   DF          Squares         Square    F Value    Pr > F

   Model                     1          2.61462        2.61462     264.08    <.0001
   Error                     5          0.04950        0.00990
   Corrected Total           6          2.66413


                Root MSE              0.09950    R-Square     0.9814
                Dependent Mean        0.32751    Adj R-Sq     0.9777
                Coeff Var            30.38189


                                Parameter Estimates

                                Parameter       Standard
        Variable     DF          Estimate          Error    t Value    Pr > |t|

        Intercept     1           0.16088        0.03898       4.13      0.0091
        LNE           1           1.21049        0.07449      16.25      <.0001

                       FREUNDLICH REGRESSION on CBHI AVERAGES
8
                                                          18:14 Saturday, October
25, 2003
```

```
         Obs    Label                 E                A      EbyA       Einv       Ainv

          1     CBHI              0.493            0.515    0.95728    2.02840    1.94175
          2     CBHI              0.737            0.703    1.04836    1.35685    1.42248
          3     CBHI              0.976            1.168    0.83562    1.02459    0.85616
          4     CBHI              1.159             1.45    0.79931    0.86281    0.68966
          5     CBHI              1.412            2.057    0.68644    0.70822    0.48614
          6     CBHI              1.827             2.41    0.75809    0.54735    0.41494
          7     CBHI              2.472            3.257    0.75898    0.40453    0.30703

         Obs       LNE         LNA      CMBLNGE      PRED        RESID

          1     -0.70725    -0.66359    1.42422    -0.69523     0.03165
          2     -0.30517    -0.35240    1.16484    -0.20852    -0.14388
          3     -0.02429     0.15529    1.01222     0.13148     0.02382
          4      0.14756     0.37156    0.92888     0.33950     0.03207
          5      0.34501     0.72125    0.84156     0.57851     0.14274
          6      0.60268     0.87963    0.73983     0.89041    -0.01079
          7      0.90503     1.18081    0.63603     1.25641    -0.07560

                         COMBINED LANGMUIR REGRESSION on CBHI AVERAGES
9
                                                                  18:14 Saturday, October
25, 2003

                                       The REG Procedure
                                         Model: MODEL1
                                    Dependent Variable: Ainv

                                      Analysis of Variance

                                              Sum of           Mean
         Source                   DF         Squares         Square    F Value    Pr > F

         Model                     1         2.07750        2.07750     129.30    <.0001
         Error                     5         0.08034        0.01607
         Corrected Total           6         2.15783


                      Root MSE             0.12676    R-Square     0.9628
                      Dependent Mean       0.87402    Adj R-Sq     0.9553
                      Coeff Var           14.50268


                                      Parameter Estimates

                                     Parameter       Standard
              Variable     DF        Estimate          Error    t Value    Pr > |t|

              Intercept     1        -1.24844        0.19271      -6.48      0.0013
              CMBLNGE       1         2.20187        0.19364      11.37      <.0001

                         COMBINED LANGMUIR REGRESSION on CBHI AVERAGES
10
                                                                  18:14 Saturday, October
25, 2003

         Obs    Label                 E                A      EbyA       Einv       Ainv

          1     CBHI              0.493            0.515    0.95728    2.02840    1.94175
          2     CBHI              0.737            0.703    1.04836    1.35685    1.42248
          3     CBHI              0.976            1.168    0.83562    1.02459    0.85616
          4     CBHI              1.159             1.45    0.79931    0.86281    0.68966
          5     CBHI              1.412            2.057    0.68644    0.70822    0.48614
          6     CBHI              1.827             2.41    0.75809    0.54735    0.41494
          7     CBHI              2.472            3.257    0.75898    0.40453    0.30703

         Obs       LNE         LNA      CMBLNGE      PRED       RESID

          1     -0.70725    -0.66359    1.42422    1.88750     0.05425
```

```
          2     -0.30517    -0.35240    1.16484    1.31638     0.10609
          3     -0.02429     0.15529    1.01222    0.98033    -0.12417
          4      0.14756     0.37156    0.92888    0.79682    -0.10717
          5      0.34501     0.72125    0.84156    0.60455    -0.11841
          6      0.60268     0.87963    0.73983    0.38056     0.03438
          7      0.90503     1.18081    0.63603    0.15201     0.15502

                        A versus E REGRESSION on AVERAGES OF FILE CBHII
11
                                                                18:14 Saturday, October
25, 2003

                                       The REG Procedure
                                         Model: MODEL1
                                     Dependent Variable: A

                                      Analysis of Variance

                                             Sum of           Mean
         Source                   DF        Squares         Square    F Value    Pr > F

         Model                     1       11.71216       11.71216     126.85    <.0001
         Error                     5        0.46165        0.09233
         Corrected Total           6       12.17380


                      Root MSE              0.30386    R-Square     0.9621
                      Dependent Mean        2.62386    Adj R-Sq     0.9545
                      Coeff Var            11.58057


                                      Parameter Estimates

                                   Parameter       Standard
              Variable     DF       Estimate          Error    t Value    Pr > |t|

              Intercept     1       -0.89267        0.33268      -2.68      0.0436
              E             1        3.07389        0.27292      11.26      <.0001

                        A versus E REGRESSION on AVERAGES OF FILE CBHII
12
                                                                18:14 Saturday, October
25, 2003

         Obs    Label                E               A      EbyA       Einv       Ainv

          1     CBHII            0.477           0.575    0.82957    2.09644    1.73913
          2     CBHII            0.715           1.073    0.66636    1.39860    0.93197
          3     CBHII            0.929           2.135    0.43513    1.07643    0.46838
          4     CBHII            1.315           2.828    0.46499    0.76046    0.35361
          5     CBHII            1.335           3.646    0.36615    0.74906    0.27427
          6     CBHII            1.463           3.782    0.38683    0.68353    0.26441
          7     CBHII            1.774           4.328    0.40989    0.56370    0.23105

         Obs       LNE         LNA      CMBLNGE      PRED       RESID

          1     -0.74024    -0.55339    1.44791    0.57357     0.00143
          2     -0.33547     0.07046    1.18262    1.30516    -0.23216
          3     -0.07365     0.75847    1.03751    1.96297     0.17203
          4      0.27384     1.03957    0.87204    3.14949    -0.32149
          5      0.28893     1.29363    0.86548    3.21097     0.43503
          6      0.38049     1.33025    0.82676    3.60443     0.17757
          7      0.57324     1.46511    0.75080    4.56041    -0.23241

                       EbyA versus E REGRESSION on AVERAGES OF FILE CBHII
13
                                                                18:14 Saturday, October
25, 2003

                                       The REG Procedure
                                         Model: MODEL1
```

```
                                   Dependent Variable: EbyA

                                      Analysis of Variance

                                             Sum of           Mean
         Source                   DF        Squares         Square    F Value    Pr > F

         Model                     1        0.13015        0.13015      13.04    0.0154
         Error                     5        0.04991        0.00998
         Corrected Total           6        0.18007


                      Root MSE              0.09991    R-Square     0.7228
                      Dependent Mean        0.50842    Adj R-Sq     0.6674
                      Coeff Var            19.65170


                                      Parameter Estimates

                                   Parameter       Standard
              Variable     DF       Estimate          Error    t Value    Pr > |t|

              Intercept     1        0.87912        0.10939       8.04      0.0005
              E             1       -0.32404        0.08974      -3.61      0.0154

                       EbyA versus E REGRESSION on AVERAGES OF FILE CBHII
14
                                                                18:14 Saturday, October
25, 2003

         Obs    Label                E                A      EbyA       Einv       Ainv

          1     CBHII            0.477            0.575    0.82957    2.09644    1.73913
          2     CBHII            0.715            1.073    0.66636    1.39860    0.93197
          3     CBHII            0.929            2.135    0.43513    1.07643    0.46838
          4     CBHII            1.315            2.828    0.46499    0.76046    0.35361
          5     CBHII            1.335            3.646    0.36615    0.74906    0.27427
          6     CBHII            1.463            3.782    0.38683    0.68353    0.26441
          7     CBHII            1.774            4.328    0.40989    0.56370    0.23105

         Obs       LNE         LNA      CMBLNGE      PRED       RESID

          1     -0.74024    -0.55339    1.44791    0.72455     0.10501
          2     -0.33547     0.07046    1.18262    0.64743     0.01893
          3     -0.07365     0.75847    1.03751    0.57809    -0.14296
          4      0.27384     1.03957    0.87204    0.45301     0.01199
          5      0.28893     1.29363    0.86548    0.44653    -0.08037
          6      0.38049     1.33025    0.82676    0.40505    -0.01822
          7      0.57324     1.46511    0.75080    0.30427     0.10562

                         LANGMUIR REGRESSION on AVERAGES OF FILE CBHII
15
                                                                18:14 Saturday, October
25, 2003

                                       The REG Procedure
                                         Model: MODEL1
                                   Dependent Variable: Ainv

                                      Analysis of Variance

                                             Sum of           Mean
         Source                   DF        Squares         Square    F Value    Pr > F

         Model                     1        1.79021        1.79021     179.32    <.0001
         Error                     5        0.04992        0.00998
         Corrected Total           6        1.84013


                      Root MSE              0.09992    R-Square     0.9729
                      Dependent Mean        0.60897    Adj R-Sq     0.9674
```

```
                      Coeff Var            16.40741


                                      Parameter Estimates

                                   Parameter       Standard
              Variable     DF       Estimate          Error    t Value    Pr > |t|

              Intercept     1       -0.44615        0.08738      -5.11      0.0038
              Einv          1        1.00787        0.07526      13.39      <.0001

                         LANGMUIR REGRESSION on AVERAGES OF FILE CBHII
16
                                                                18:14 Saturday, October
25, 2003

         Obs    Label                E               A      EbyA       Einv       Ainv

          1     CBHII            0.477           0.575    0.82957    2.09644    1.73913
          2     CBHII            0.715           1.073    0.66636    1.39860    0.93197
          3     CBHII            0.929           2.135    0.43513    1.07643    0.46838
          4     CBHII            1.315           2.828    0.46499    0.76046    0.35361
          5     CBHII            1.335           3.646    0.36615    0.74906    0.27427
          6     CBHII            1.463           3.782    0.38683    0.68353    0.26441
          7     CBHII            1.774           4.328    0.40989    0.56370    0.23105

         Obs       LNE         LNA      CMBLNGE      PRED       RESID

          1     -0.74024    -0.55339    1.44791    1.66678     0.07235
          2     -0.33547     0.07046    1.18262    0.96346    -0.03149
          3     -0.07365     0.75847    1.03751    0.63875    -0.17036
          4      0.27384     1.03957    0.87204    0.32029     0.03332
          5      0.28893     1.29363    0.86548    0.30881    -0.03454
          6      0.38049     1.33025    0.82676    0.24276     0.02165
          7      0.57324     1.46511    0.75080    0.12198     0.10907

                        FREUNDLICH REGRESSION on AVERAGES OF FILE CBHII
17
                                                                18:14 Saturday, October
25, 2003

                                       The REG Procedure
                                         Model: MODEL1
                                    Dependent Variable: LNA

                                      Analysis of Variance

                                             Sum of           Mean
         Source                   DF        Squares         Square    F Value    Pr > F

         Model                     1        3.29073        3.29073     175.06    <.0001
         Error                     5        0.09399        0.01880
         Corrected Total           6        3.38472


                      Root MSE              0.13711    R-Square     0.9722
                      Dependent Mean        0.77201    Adj R-Sq     0.9667
                      Coeff Var            17.75953


                                      Parameter Estimates

                                   Parameter       Standard
              Variable     DF       Estimate          Error    t Value    Pr > |t|

              Intercept     1        0.68787        0.05221      13.18      <.0001
              LNE           1        1.60434        0.12126      13.23      <.0001

                        FREUNDLICH REGRESSION on AVERAGES OF FILE CBHII
18
                                                                18:14 Saturday, October
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```
25, 2003

         Obs    Label                E               A      EbyA       Einv       Ainv

          1     CBHII            0.477           0.575    0.82957    2.09644    1.73913
          2     CBHII            0.715           1.073    0.66636    1.39860    0.93197
          3     CBHII            0.929           2.135    0.43513    1.07643    0.46838
          4     CBHII            1.315           2.828    0.46499    0.76046    0.35361
          5     CBHII            1.335           3.646    0.36615    0.74906    0.27427
          6     CBHII            1.463           3.782    0.38683    0.68353    0.26441
          7     CBHII            1.774           4.328    0.40989    0.56370    0.23105

         Obs       LNE         LNA      CMBLNGE      PRED        RESID

          1     -0.74024    -0.55339    1.44791    -0.49972    -0.05366
          2     -0.33547     0.07046    1.18262     0.14966    -0.07920
          3     -0.07365     0.75847    1.03751     0.56972     0.18875
          4      0.27384     1.03957    0.87204     1.12720    -0.08763
          5      0.28893     1.29363    0.86548     1.15141     0.14222
          6      0.38049     1.33025    0.82676     1.29830     0.03195
          7      0.57324     1.46511    0.75080     1.60753    -0.14243

                     COMBINED LANGMUIR REGRESSION on AVERAGES OF FILE CBHII
19
                                                                18:14 Saturday, October
25, 2003

                                       The REG Procedure
                                         Model: MODEL1
                                   Dependent Variable: Ainv

                                      Analysis of Variance

                                             Sum of           Mean
         Source                  DF         Squares         Square    F Value    Pr > F

         Model                    1         1.73091        1.73091      79.24    0.0003
         Error                    5         0.10922        0.02184
         Corrected Total          6         1.84013


                      Root MSE             0.14779    R-Square     0.9406
                      Dependent Mean       0.60897    Adj R-Sq     0.9288
                      Coeff Var           24.26932


                                      Parameter Estimates

                                   Parameter       Standard
              Variable     DF       Estimate          Error    t Value    Pr > |t|

              Intercept     1       -1.57266        0.25136      -6.26      0.0015
              CMBLNGE       1        2.18691        0.24567       8.90      0.0003

                     COMBINED LANGMUIR REGRESSION on AVERAGES OF FILE CBHII
20
                                                                18:14 Saturday, October
25, 2003

         Obs    Label                E               A      EbyA       Einv       Ainv

          1     CBHII            0.477           0.575    0.82957    2.09644    1.73913
          2     CBHII            0.715           1.073    0.66636    1.39860    0.93197
          3     CBHII            0.929           2.135    0.43513    1.07643    0.46838
          4     CBHII            1.315           2.828    0.46499    0.76046    0.35361
          5     CBHII            1.335           3.646    0.36615    0.74906    0.27427
          6     CBHII            1.463           3.782    0.38683    0.68353    0.26441
          7     CBHII            1.774           4.328    0.40989    0.56370    0.23105

         Obs       LNE         LNA      CMBLNGE      PRED       RESID
```

```
          1     -0.74024    -0.55339    1.44791    1.59378     0.14535
          2     -0.33547     0.07046    1.18262    1.01363    -0.08166
          3     -0.07365     0.75847    1.03751    0.69628    -0.22789
          4      0.27384     1.03957    0.87204    0.33441     0.01919
          5      0.28893     1.29363    0.86548    0.32008    -0.04580
          6      0.38049     1.33025    0.82676    0.23538     0.02903
          7      0.57324     1.46511    0.75080    0.06927     0.16179

                         A versus E REGRESSION on AVERAGES OF FILE EGII
21
                                                                18:14 Saturday, October
25, 2003

                                       The REG Procedure
                                         Model: MODEL1
                                     Dependent Variable: A

                                      Analysis of Variance

                                             Sum of           Mean
         Source                   DF        Squares         Square    F Value    Pr > F

         Model                     1       10.67175       10.67175     159.84    <.0001
         Error                     5        0.33382        0.06676
         Corrected Total           6       11.00557


                      Root MSE              0.25839    R-Square     0.9697
                      Dependent Mean        2.22086    Adj R-Sq     0.9636
                      Coeff Var            11.63465


                                      Parameter Estimates

                                   Parameter       Standard
              Variable     DF       Estimate          Error    t Value    Pr > |t|

              Intercept     1       -0.44148        0.23213      -1.90      0.1156
              E             1        2.14928        0.17000      12.64      <.0001

                         A versus E REGRESSION on AVERAGES OF FILE EGII
22
                                                                18:14 Saturday, October
25, 2003

         Obs    Label                E               A      EbyA       Einv       Ainv

          1     EGII             0.362           0.178    2.03371    2.76243    5.61798
          2     EGII              0.69            1.07    0.64486    1.44928    0.93458
          3     EGII             0.997           1.928    0.51712    1.00301    0.51867
          4     EGII              1.14            1.82    0.62637    0.87719    0.54945
          5     EGII             1.556           3.135    0.49633    0.64267    0.31898
          6     EGII             1.857            3.76    0.49388    0.53850    0.26596
          7     EGII             2.069           3.655    0.56607    0.48333    0.27360

         Obs       LNE         LNA      CMBLNGE      PRED       RESID

          1     -1.01611    -1.72597    1.66206    0.33656    -0.15856
          2     -0.37106     0.06766    1.20386    1.04152     0.02848
          3     -0.00300     0.65648    1.00150    1.70135     0.22665
          4      0.13103     0.59884    0.93659    2.00869    -0.18869
          5      0.44212     1.14263    0.80167    2.90279     0.23221
          6      0.61896     1.32442    0.73383    3.54972     0.21028
          7      0.72707     1.29610    0.69522    4.00537    -0.35037

                       EbyA versus E REGRESSION on AVERAGES OF FILE EGII
23
                                                                18:14 Saturday, October
25, 2003

                                       The REG Procedure
```

```
                             Model: MODEL1
                       Dependent Variable: EbyA

                          Analysis of Variance

                                   Sum of           Mean
Source                   DF       Squares         Square    F Value    Pr > F

Model                     1       0.83730        0.83730       3.98    0.1027
Error                     5       1.05259        0.21052
Corrected Total           6       1.88990


             Root MSE              0.45882    R-Square     0.4430
             Dependent Mean        0.76834    Adj R-Sq     0.3317
             Coeff Var            59.71653


                          Parameter Estimates

                         Parameter       Standard
     Variable     DF      Estimate          Error    t Value    Pr > |t|

     Intercept     1       1.51408        0.41219       3.67      0.0144
     E             1      -0.60203        0.30187      -1.99      0.1027

               EbyA versus E REGRESSION on AVERAGES OF FILE EGII
24
                                                     18:14 Saturday, October
25, 2003

Obs    Label                E               A      EbyA       Einv       Ainv

 1     EGII             0.362           0.178    2.03371    2.76243    5.61798
 2     EGII              0.69            1.07    0.64486    1.44928    0.93458
 3     EGII             0.997           1.928    0.51712    1.00301    0.51867
 4     EGII              1.14            1.82    0.62637    0.87719    0.54945
 5     EGII             1.556           3.135    0.49633    0.64267    0.31898
 6     EGII             1.857            3.76    0.49388    0.53850    0.26596
 7     EGII             2.069           3.655    0.56607    0.48333    0.27360

Obs       LNE         LNA      CMBLNGE      PRED       RESID

 1     -1.01611    -1.72597    1.66206    1.29614     0.73757
 2     -0.37106     0.06766    1.20386    1.09868    -0.45382
 3     -0.00300     0.65648    1.00150    0.91385    -0.39674
 4      0.13103     0.59884    0.93659    0.82776    -0.20139
 5      0.44212     1.14263    0.80167    0.57732    -0.08099
 6      0.61896     1.32442    0.73383    0.39611     0.09777
 7      0.72707     1.29610    0.69522    0.26848     0.29759

                  LANGMUIR REGRESSION on AVERAGES OF FILE EGII
25
                                                     18:14 Saturday, October
25, 2003

                           The REG Procedure
                             Model: MODEL1
                       Dependent Variable: Ainv

                          Analysis of Variance

                                   Sum of           Mean
Source                   DF       Squares         Square    F Value    Pr > F

Model                     1      20.86224       20.86224      49.20    0.0009
Error                     5       2.12011        0.42402
Corrected Total           6      22.98235


             Root MSE              0.65117    R-Square     0.9078
```

```
                Dependent Mean        1.21132    Adj R-Sq     0.8893
                Coeff Var            53.75724


                                Parameter Estimates

                             Parameter       Standard
        Variable     DF       Estimate          Error    t Value    Pr > |t|

        Intercept     1       -1.36839        0.44253      -3.09      0.0271
        Einv          1        2.32813        0.33191       7.01      0.0009

                       LANGMUIR REGRESSION on AVERAGES OF FILE EGII
26
                                                               18:14 Saturday, October
25, 2003

         Obs    Label              E              A      EbyA       Einv       Ainv

          1     EGII           0.362          0.178    2.03371    2.76243    5.61798
          2     EGII            0.69           1.07    0.64486    1.44928    0.93458
          3     EGII           0.997          1.928    0.51712    1.00301    0.51867
          4     EGII            1.14           1.82    0.62637    0.87719    0.54945
          5     EGII           1.556          3.135    0.49633    0.64267    0.31898
          6     EGII           1.857           3.76    0.49388    0.53850    0.26596
          7     EGII           2.069          3.655    0.56607    0.48333    0.27360

         Obs       LNE          LNA      CMBLNGE      PRED        RESID

          1     -1.01611    -1.72597    1.66206     5.06291     0.55506
          2     -0.37106     0.06766    1.20386     2.00571    -1.07113
          3     -0.00300     0.65648    1.00150     0.96675    -0.44807
          4      0.13103     0.59884    0.93659     0.67383    -0.12438
          5      0.44212     1.14263    0.80167     0.12784     0.19114
          6      0.61896     1.32442    0.73383    -0.11468     0.38064
          7      0.72707     1.29610    0.69522    -0.24315     0.51674

                      FREUNDLICH REGRESSION on AVERAGES OF FILE EGII
27
                                                               18:14 Saturday, October
25, 2003

                                 The REG Procedure
                                   Model: MODEL1
                              Dependent Variable: LNA

                                Analysis of Variance

                                        Sum of           Mean
         Source                   DF       Squares         Square    F Value    Pr > F

         Model                     1       6.46735        6.46735      74.79    0.0003
         Error                     5       0.43239        0.08648
         Corrected Total           6       6.89974


                Root MSE              0.29407    R-Square     0.9373
                Dependent Mean        0.48002    Adj R-Sq     0.9248
                Coeff Var            61.26222


                                Parameter Estimates

                             Parameter       Standard
        Variable     DF       Estimate          Error    t Value    Pr > |t|

        Intercept     1        0.35203        0.11213       3.14      0.0257
        LNE           1        1.69368        0.19585       8.65      0.0003

                      FREUNDLICH REGRESSION on AVERAGES OF FILE EGII
28
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                                                                18:14 Saturday, October
25, 2003

         Obs    Label                E                A      EbyA       Einv       Ainv

          1     EGII             0.362            0.178    2.03371    2.76243    5.61798
          2     EGII              0.69             1.07    0.64486    1.44928    0.93458
          3     EGII             0.997            1.928    0.51712    1.00301    0.51867
          4     EGII              1.14             1.82    0.62637    0.87719    0.54945
          5     EGII             1.556            3.135    0.49633    0.64267    0.31898
          6     EGII             1.857             3.76    0.49388    0.53850    0.26596
          7     EGII             2.069            3.655    0.56607    0.48333    0.27360

         Obs       LNE         LNA      CMBLNGE      PRED        RESID

          1     -1.01611    -1.72597    1.66206    -1.36893    -0.35704
          2     -0.37106     0.06766    1.20386    -0.27643     0.34409
          3     -0.00300     0.65648    1.00150     0.34694     0.30954
          4      0.13103     0.59884    0.93659     0.57395     0.02489
          5      0.44212     1.14263    0.80167     1.10083     0.04179
          6      0.61896     1.32442    0.73383     1.40035    -0.07593
          7      0.72707     1.29610    0.69522     1.58344    -0.28735

                     COMBINED LANGMUIR REGRESSION on AVERAGES OF FILE EGII
29
                                                                18:14 Saturday, October
25, 2003

                                       The REG Procedure
                                         Model: MODEL1
                                   Dependent Variable: Ainv

                                      Analysis of Variance

                                             Sum of           Mean
         Source                   DF        Squares          Square    F Value    Pr > F

         Model                     1       19.04212       19.04212      24.16    0.0044
         Error                     5        3.94024        0.78805
         Corrected Total           6       22.98235


                      Root MSE              0.88772    R-Square     0.8286
                      Dependent Mean        1.21132    Adj R-Sq     0.7943
                      Coeff Var            73.28558


                                      Parameter Estimates

                                   Parameter       Standard
              Variable     DF       Estimate          Error    t Value    Pr > |t|

              Intercept     1       -4.08032        1.12756      -3.62      0.0152
              CMBLNGE       1        5.26552        1.07117       4.92      0.0044

                     COMBINED LANGMUIR REGRESSION on AVERAGES OF FILE EGII
30
                                                                18:14 Saturday, October
25, 2003

         Obs    Label                E                A      EbyA       Einv       Ainv

          1     EGII             0.362            0.178    2.03371    2.76243    5.61798
          2     EGII              0.69             1.07    0.64486    1.44928    0.93458
          3     EGII             0.997            1.928    0.51712    1.00301    0.51867
          4     EGII              1.14             1.82    0.62637    0.87719    0.54945
          5     EGII             1.556            3.135    0.49633    0.64267    0.31898
          6     EGII             1.857             3.76    0.49388    0.53850    0.26596
          7     EGII             2.069            3.655    0.56607    0.48333    0.27360

         Obs       LNE         LNA      CMBLNGE      PRED        RESID
```

```
1     -1.01611    -1.72597    1.66206     4.67127     0.94671
2     -0.37106     0.06766    1.20386     2.25862    -1.32404
3     -0.00300     0.65648    1.00150     1.19312    -0.67445
4      0.13103     0.59884    0.93659     0.85129    -0.30184
5      0.44212     1.14263    0.80167     0.14089     0.17809
6      0.61896     1.32442    0.73383    -0.21633     0.48229
7      0.72707     1.29610    0.69522    -0.41964     0.69324
```