A AMYLASE ECONOMIC AND APPLICATION VALUE

Nagib A. Elmarzugi1,2,3, Hesham A. El Enshasy1, Mariani AbdulHamid1, Rosmani Hasham1, Azila Aziz1, Elsayed A. Elsayed4, Nor Zalina Othman1, Mohamed Salama5,*

1Institute of Bioproduct Development (IBD), Universiti Teknologi Malaysia (UTM), 81310 Skudai, Johor, Malaysia.
2Dept. of Industrial Pharmacy, Faculty of Pharmacy, Tripoli University, Tripoli, Libya.
3BioNano Integration Research Group, Biotechnology Research Center, NASR, Libya.
4Bioproducts Research Chair, Zoology Department, Faculty of Science, King Saud University (KSU), Kingdom of Saudi Arabia.
5Faculty of Pharmacy, Universiti Teknologi Mara, Malaysia

ABSTRACT

The years of the research in biotechnology has boosted the importance of enzymes in industry, as well as advanced characteristics of using bioprocess rather than chemical synthesis in their preparation. Enzymes are important in biological processes because they accelerate specific biochemical reactions to produce a useful product or effect. Enzymes are widely used in industrial processes due to their low cost, large productivity and vast availability. Amylases are one of the most important enzymes in present-day biotechnology. Amylases contribute to world enzyme market about 25-30%. The estimated value of world market of amylase industry is about US$ 2.7 billion at 2012 and estimated to increase by 4% annually. α-amylase has variable wide applications. Textile de-sizing based on thermo-stable bacterial amylase covers about 12% of amylase market. α-amylase uses a very small quantity of bio-catalysis in bio-washing products instead of very high quantity of chemical detergents. In food production, enzymes are preferred to alternate the chemical-based technology. α-amylase is strongly integrated with medical and pharmaceutical applications. These applications include therapeutic and diagnostic tools for managing a quite number of diseases ranging from ordinary problems to gene therapy by correcting the enzyme deficiency.
Key Words: Enzyme, α-amylase, textile de-sizing, bio-washing, Starch.

INTRODUCTION
Enzymes play a key role in almost all biological processes, accelerating a variety of metabolic reactions as well as controlling the energy for genetic information pathways. Enzymes are biological catalysts responsible for supporting almost all of the chemical reactions that maintain general homeostasis; because of their role in maintaining life processes (Kamerlin and Warshel 2010). They are tools of nature and using natures’ own technology in modern industries, associated with many advantages, compared to chemicals, enzymes are specific in their action and work at mild conditions (M., S. et al. 1996). The enzyme is also called Biocatalyst in order to catalyse any reaction. Biocatalysts are vitally important in the industrial process to be fully exploited. Biocatalysts can be either intact cells or isolated enzymes (Pisliakov, Cao et al. 2009). The special properties of biocatalysts include conversion of substrates into the desired products both in single reactions and in complex cell systems, synthesis of products under mild reaction conditions, which approximate ambient conditions with regard to pressure, temperature and pH value. Therefore, using of enzymes in bioprocess industry could offer a number of advantages such as thirty percent saving in raw materials during production process, and up to thirty percent energy savings with mechanical process in production (Bonnet and Millet 1996). Enzymes are proteins, composed of hundreds of amino acids, which are produced by living organisms. They are responsible for number of reaction and biological activities in plant, animal, human beings and microorganisms. They are found in the human digestive system to break down carbohydrates (sugars), fats or proteins present in food. The smaller pieces can be absorbed into the blood stream; each 10-25% decrease in chlorine chemicals by substituting Xylanase before the first bleaching step. Moreover, the use of this method in the European paper industry could cut annual CO₂ emissions by 155,000 to 270,000 tons. Therefore, reduction in the use of chemicals, e.g. chlorine, EDTA and peroxides, by enzymatic deinking of recycled paper by lipase, cellulases, xylanases or pectinases. The economical paper production by efficient use of recycling paper and more protection of the environment by reducing the volume of waste paper and the demand for wood (Namvar-Mahboub and Pakizeh 2012). Enzymes are catalysts and most of them are protein and few ribonucleoprotein enzymes have been discovered and, for some of these, the catalytic activity is in the RNA part rather than the protein part (C., Q. et al. 2011). Enzymes bind temporarily to one or more of the
reactants of the reaction they catalyze. In doing so, they lower the amount of activation energy needed and thus speed up the reaction, which is represented in figure 1.

![Figure1. Effect of activation energy on chemical reaction.](image)

**History of enzymes industry**

The history of modern enzyme technology could be traced to the beginning of 1874 when Hansen manufactured Chymosin from the stomach of calves for manufacturing of cheese. This was the first enzyme preparation of relatively high purity used for industrial purposes. Jokichi Takamine was the first person to manufacture an enzyme from a microbial source when he manufactured taka diastase from Aspergillus as a digestive enzyme in 1894. The method of fermentation suggested by Takamin, the "surface culture" is still actively used in the production of various enzymes. Bacterial amylase derived from *Bacillus Subtilis* was used for desizing, the first time by Boidin and Effront as early as 1917. In 1950, fungal amylase was used in the manufacture of specific types of syrup, those containing a range of sugars, which could not be produced by conventional acid hydrolysis. The real turning point was reached early in 1960, when an enzyme glucoamylase was launched for the first time, which could completely break down starch into glucose. The process was further improved by the introduction of a new technique used for the enzymatic pre-treatment of starch by using a heat stable alpha amylase (Johnson 2013).

**Enzyme cofactors**

Numerous enzymes require the presence of an additional, non-protein and co-factor. Many of these are metal ions such as Zn$^{2+}$ that act as co-factor for carbonic anhydrase, Cu$^{2+}$, Mn$^{2+}$, K$^+$, and Na$^+$. Some cofactors are small organic molecules called coenzymes. The B vitamins
[thiamine (B1), riboflavin (B2) and nicotinamide] are precursors of coenzymes (Sennett, Kadirvelraj et al. 2012).

In the last few years the time for molecular biological optimization of enzymes has decreased by a factor of 10-100, while at the same time the stability and productivity of selected enzymes has unmistakably risen. In the past ten years, the market volume for enzymes rose by approximately 50 %. A US study made in 2004 anticipates annual growth of the US-American enzyme market of 6 % and a market volume of US$ 1.9 billion by 2008, the percentage of total applications of enzymes by market sectors (Flaschel E, Bott M et al. 2006).

Table1. Distribution of the worldwide application of technical enzymes by market sectors (Flaschel E, Bott M et al. 2006)

<table>
<thead>
<tr>
<th>Market sector</th>
<th>% share of total application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detergent</td>
<td>40</td>
</tr>
<tr>
<td>Cheese ripening and aroma</td>
<td>12</td>
</tr>
<tr>
<td>Flour and baking products</td>
<td>10</td>
</tr>
<tr>
<td>Leather processing</td>
<td>10</td>
</tr>
<tr>
<td>Glucose isomerization</td>
<td>7</td>
</tr>
<tr>
<td>Fruit utilization and wine</td>
<td>7</td>
</tr>
<tr>
<td>Starch degradation</td>
<td>5</td>
</tr>
<tr>
<td>Brewery (not in Germany)</td>
<td>5</td>
</tr>
<tr>
<td>Silage and feed</td>
<td>2</td>
</tr>
<tr>
<td>Pulp and paper, textile</td>
<td>2</td>
</tr>
</tbody>
</table>

Structure

According to computer modular simulation studies, the protein engineering of amylase enzyme found in human saliva and pancreas. This enzyme can digest starch molecules and break them down to sugar molecules. Enzyme is made of a sequence of amino acids folded into a unique three-dimensional structure that determines the function of the enzyme. Even the slightest change in the sequence of the amino acids can alter the shape and function of the enzyme. Each enzyme consists of several hundreds of amino acids located in such a delicate three-dimensional structure as in Figure 2 and 3. This structure determines the properties of the enzyme such as reactivity, stability and specificity. Enzyme are essential for all metabolic processes, but are distinguishable from other proteins because they are known as biological catalysts or substances, which speed up reaction without get used up themselves.
Production of enzymes

Enzyme molecules are too complex to synthesize by purely chemical means. The problem is that enzyme produced by microorganisms often expressed in tiny amount mixed up with many other enzyme and proteins. These microorganisms can also be very difficult to cultivate under industrial condition, and they may create undesirable by-product. Modern industrial cultivation of enzymes begins with fermentation of a vial of dried or frozen microorganisms called a production strain. This production strain is selected to produce large amounts of the enzymes of interest. The production strain is first cultivated in a small flask containing nutrients and a gar, the flask placed in an incubator, which provides the optimal temperature for the previously frozen or dried cells to germinate. Once the flask is ready, the cells are transferred to a seed fermented, which is a large tank containing previously sterilized raw materials and water, known as the medium, seed fermentation allows the cell to reproduce and adapt to the environment and nutrients that they will encounter later on. The cells are then transferred to a larger tank, the main fermented, where temperature, pH and dissolved oxygen are carefully controlled to optimize enzyme production, and additional nutrients may be added to enhance productivity. Therefore, when main fermentation is complete the mixture of cells, nutrient and enzyme, referred to as the broth, is ready for filtration and purification.

Amylase Enzyme

Microbial enzymes are widely used in industrial processes due to their low cost, large productivity, chemical stability, environmental protection, plasticity and vast availability (N. Sajitha, V. Vasanthabharathi et al. 2011, Yasser Bakri, Hassan Ammouneh et al. 2012). Bacillus species such as Bacillus subtilis, Bacillus amyloliquefaciens and Bacillus
lichenumformis are used as bacterial workhorses in industrial microbial cultivations for the production of a variety of enzymes as well as fine biochemicals for decades. A large quantity (20-25g/l) of extracellular enzymes has been produced and secreted by the various Bacillus strains which have placed them among the most significant industrial enzyme producers. The estimated value of world market is presently about US$ 2.7 billion and is estimated to increase by 4% annually through 2012. Detergents (37%), textiles (12%), starch (11%), baking (8%) and animal feed (6%) are the main industries, which use about 75% of industrially produced enzymes (Nik C. Barbet, Ulrich Schneider et al. 1996, C. Meintanis, K.I. Chalkou et al. 2008). Amylases are among the most important enzymes having great significance for biotechnology, constituting a class of industrial enzymes having approximately 25-30% of the world enzyme market (Deb, Talukdar et al. 2013). Initially the term amylase was used originally to designate an extracellular enzymes capable of hydrolyzing α-1,4-glucosidic linkages in polysaccharides containing three or more 1,4-α-linked glucose units. The enzyme acts on starches, glycogen and oligosaccharides in a random manner, liberating reducing groups. These enzymes are found in prokaryote as well as in eukaryotic organisms. They are widely distributed in microbial, plant and animal kingdoms (H., S. et al. 2013). Bacterial growth requires a source of energy in order to assemble the various constituents comprising the cell. The energy supply is derived from the controlled breakdown of various organic substrates present in the external environment, such as polysaccharides, lipids, and proteins. Some members of the genus Bacillus are able to break down starch and utilize it as an energy source. Organisms unable to produce amylase cannot utilize starch as an energy source (Rothstein, Devlin et al. 1986, Pretorius I, Laing E et al. 1988). Amylases are classified based on how they break down starch molecules into: I- α-amylase (alpha-amylase) which reduces the viscosity of starch by breaking down the bonds at random, therefore producing varied sized chains of glucose. II- β-amylase (Beta-amylase) which breaks the glucose-glucose bonds down by removing two glucose units at a time, thereby producing maltose. III- Amyloglucosidase (AMG), which breaks successive, bonds from the non reducing end of the straight chain, producing glucose (Carugo, Lu et al. 2001). The Amylase enzyme has a shape that allows it to wrap around starch (substrate) and cut it up into individual glucose units (Pandey A, Nigam P et al. 2000). α-amylase is an endo acting enzyme, catalyzing the random hydrolysis of internal α1 to 4 glycosidic linkages present in the starch substrate. These enzymes are incapable of hydrolysing α1 to 6 glycosidic linkages present at branch points of amylopectin chains. One exception to this is the α-amylase produced by thermoactinomyces vulgaris, which can hydrolyse both α1 to 6 and α1 to 4
glycosidic linkages (Vihinen and Mantiiila 1989). α-amylase has been derived from several fungi, yeasts, bacteria and actinomycetes, however, enzymes from fungal and bacterial sources have dominated applications in industrial sectors. Such bulk enzymes are produced primarily by bacteria by members of the general Bacillus. Fungal α-amylases most commonly used industrially are produced by species of Aspergillus, most notably A. oryzae and only one species of Penicillium is Penicillium fellutanum (Flaschel E, Bott M et al. 2006). A few industrially important enzymes may be obtained from yeast, mainly from species of Saccharomyces. Amylase growth and production by Bacillus sp. WN11 was studied under different cultivation conditions. Maximum growth was observed at 65 °C while the highest amylase production was recorded at 55 °C. Accounted amylase production and growth was observed at an initial medium pH of 5 and 6. Growth was not detected, and observed at pH 4 and 9. The organism grew better and produced higher levels of amylase activity using proteose peptone and tryptone as nitrogen sources. Growth was not observed, when NO3+, NH4+ and urea were used as nitrogen sources. Among defined carbohydrates, tested starch and maltose supported good growth and amylase production, with the highest productivity recorded in the presence of starch (24 U/ml) (Mamo and Gessesse 1999).

**Amylase substrate**

The starch as substrate represents the most abundant storage of polysaccharides in plants, and next to cellulose, is the most abundant polysaccharide found on earth. The starch polymer consists exclusively of glucose units. Two forms exist, namely α-amylose and amyllopectin. α-amylose is along, linear polymer in which successive D-glucose molecules are linked by an α 1 to 4 glycosidic bond. Individual α-amylose chains may vary in length and hence in molecular masses in the region of 500 kDa. Abundant supplies of starch may obtain from seeds and tubers, such as corn, wheat, rice, tapioca and potato. The widespread availability of starch from such inexpensive sources, coupled with large-scale production of amylolytic enzymes, facilitates production of syrups, containing glucose, fructose or maltose, which is considerable importance in the food and confectionery industry (Elmarzugi, Enshasy et al. 2010). The initial step in starch hydrolysis entails disruption of the starch granule. Solubilization of the granules, the process of “gelatinization” facilitates subsequent catalytic degradation. Gelatinization is normally, achieved by heating the starch slurry to temperatures in excess of 100°C for several minutes. α-amylase may be added immediately prior to the heating step, in order to render more efficient the process of granules disruption. Once the
granules have been disrupted, additional α-amylase is added in order to liquefy the starch. This process reduces the viscosity of the starch solution, and this can be shown in figure 4.

![Diagram of starch hydrolysis process](image)

**Figure 4. Controlled hydrolysis of starch, yielding up to a 96% glucose syrup.**

Starch may be enzymatically hydrolysed using varying combination of amylolytic enzymes in order to generate specific end products. The major end products produced commercially are maltodextrins or corn syrup solids, maltose syrup, glucose syrup or high fructose corn syrup, and cyclodextrins. Maltodextrins are produced by partial enzymatic hydrolysis of starch, using α-amylase. The product is often defined as a non sweet nutritive saccharide mix, largely containing glucose units, which is linked primarily by α1 to 4 glycosidic linkages. Although the exact product composition can vary, it generally consists of glucose (approx. 1%), maltose (3-5%), maltotriose (5-10%), maltotetraose (6%), with the reminder (75% or more) being saccharides of higher molecular mass (Rodríguez, Alameda et al. 2006). When the desired degree of starch hydrolysis attained by α-amylase, the pH value of the slurry dropped from 6.5 to 3.0 and the slurry, is then heated to boiling for about five minutes in order to totally inactivate the α-amylase. Filtration and passage through a carbon column removes any particulates and colour, and the resultant purified maltodextrin mix is evaporated and subsequently spray dried to yield a powdered product. The reaction rates of amylase and starch increased as the temperature increased. The absorbance levels of the starch decreased as the temperature increased. However, after 65 degree trial, the reaction rates starts to decrease and the absorbance levels increased (Nahas E and Waldemarin MM 2012).
Starch digestion in humans
The action of α-amylase on starch in the human body is digesting starch and producing shorter chain glucose polymers, which are very similar to the action of α-amylase outside human body. In humans, the salivary and pancreatic glands produce α-amylase. However, the action of salivary amylase is limited to the hydrolysis of starch to lower degree of polymerization polymers. The principal hydrolysis of starch by pancreatic α-amylase occurs within the lumen of the small intestine; this action produces shorter chain polymers of lower average polymerization degree than the intact starch molecule. The lower degree of polymerization polymers are subsequently act on by other enzymes, including glucoamylase, which cleaves the polymers into individual glucose monomers residues that are absorbed by the body (Ai, Nelson et al. 2013, Elmarzugi, Enshasy et al. 2013).

The impact of genetic engineering
Genetic engineering continues to have a major impact upon the industrial enzyme sector. Throughout the 1980s the major enzyme companies initiated research programmed aimed at producing selected industrial enzymes by recombinant technology. Recombinant strategies using both homologous and heterologous expression systems. Production of industrial enzymes by recombinant means displays a number of potential advantages, as compared to traditional (non-recombinant) approaches. These advantages may include the higher expression levels attainable; product generally displays a higher degree of relative purity. Moreover, it is considered economically attractive. Heterologous expression facilities commercialization of enzymes produced naturally by pathogenic/ non-GRAS-listed species, also, allows alteration of enzyme’s characteristics via protein engineering. The advent of recombinant DNA technology has facilitated the cloning and expression of genes coding for various α-amylases in a variety of recombinant organisms. Human, wheat and bacterial α-amylases, for example, been expressed in saccharomyces cerevisiae. More recently, the gene coding for B. licheniformis α-amylase has been expressed in transgenic tobacco plants, this was among the first examples of the production of bulk industrial enzymes in a recombinant plant species. Microbial amylases could be potentially useful in the pharmaceutical and fine chemical industries if enzymes with suitable properties could be prepared. The molecular mass of the recombinant protein was found to be 64 kDa, compared to 55 kDa in the native Bacillus species (Deb, Talukdar et al. 2013).
Applications

α-Amylase in textile de-sizing

Initial moves to apply biotechnology have been predominantly in textile finishing. Biotechnological processes have already proved more advantageous than chemical processes in the processing of natural fibres, such as cotton and wool. Modern methods of textile weaving, places considerable mechanical stress on the fabric threads to prevent breakage, the fabric strands are nearly always coated with a substance known as a ‘size’. The serves to strengthen the fabric prior to weaving. Essential attributes of sizing materials include good adhesion to the textile threads, ease of removal after weaving, it is necessary to remove the size after weaving as it would subsequently prevent proper dyeing or bleaching of the finished product and inexpensiveness. Subsequent removal of the starch (i.e. ‘desizing’) may be achieved by steam heating in presence of NaOH, or by oxidants. However, such treatments can damage the textile and will generate a process effluent which must treat before disposal. Desizing using α-amylase has thus become popular. Generally, thermo-stable bacterial α-amylases (e.g. B. licheniformis α-amylase) are mainly used. Depending upon the enzyme concentration and environmental parameters chosen, the desizing process may last from several minutes to several hours (Walsh G., 2005; Flashel E. et al., 2006). The European textile industry is exposed to fierce international competition and price wars. In the last few years this has caused parts of the European textile production to migrate to Asia. As the European textile industry cannot compete with Asian complying with respect to wages and the cost of complying with environmental restrictions it is dependent on initiating new products and new production processes, for example technical textiles (textiles with customized and functional properties). High growth rates are particularly anticipated for technical and functional textiles, e.g. textiles for persons with allergies, antimicrobial textiles, textiles for hospital hygiene and those incorporated into cosmetics and pharmaceuticals. Like textiles, pulp and paper are also often sized using starch. Sizing of paper protects it from mechanical damage during manufacture, and enhances the stiffness and feel of the finished product, in this case desizing is not subsequently carried out. Natural unprocessed starch slurries are too viscous to be used in paper sizing. α-amylase is used to partially degrade the starch in order to yield a product of appropriate viscosity for the task at hand. The world market for enzymes for use in the pulp and paper industry has a volume of around € 10m and an estimated annual growth 25 % (Longyun Hao, Rui Wang et al. 2013). As biotechnological processes have proved more advantageous than chemical processes, 80 % of the European textile industry now uses the biocatalyst amylase to remove starch from natural
fibres (e.g. cotton) during the desizing process. In various areas of textile industry the increasing application of biotechnological processes as in sourcing cotton has enabled the branch in the short to medium term to produce more competitively and with less pollution and to develop more innovative products (Flaschel E, Bott M et al. 2006).

**α-Amylase in detergency (bio-washing)**

Enzyme is used in cleaning product as cleaning and fabric care agent. Most of the used of enzyme types breakdown large water insoluble soils and stain which attached to fabrics into smaller, therefore removed by mechanical action of the washing machine or by interaction with other detergent agent. Enzyme does not loose its functionality after having worked on one stain and continues to work on the next one. The most important reason to apply enzyme in **detergency is the use of** very small quantity of these bio-catalysts that can replace very large quantity of chemical detergent (Mitidieri, Souza Martinelli et al. 2006). Amylases are used to remove residues of starch-based foods like potatoes, spaghetti, custards, gravies and chocolate. This type of enzyme can be used in laundry detergency as well as in dishwashing detergency. α-amylases have been used in detergents since the beginning of the 1970s and the demand is still increasing. The use of a such enzyme could reduce energy consumption, since washing cycles can be carried out at 30-40°C instead of 80-90°C and less cycles are necessary, and consequently reduced water consumption (up to 50%). Moreover, a distinguish saving by enzymatic removal of impurities from cotton fibres, and by using catalases for hydrogen peroxide removal after bleaching, so a decrease in the amount of chemicals used and a decrease in the pollution due to reduced wastewater (Roy, Rai et al. 2012). The detergent α-amylases in use today derived mainly from the Bacillus family, have highly homologous primary amino acid sequences and a tertiary structure comprised of three domains A, B and C. The active site is typically located in a cleft between domains A and B and is usually comprised of acidic amino acids such as aspartic and glutamic acids. Like many proteases, most α-amylases contain essential calcium ions, which are important in maintaining the tertiary structure of the enzyme molecule. In 2004, a new amylase, Stainzyme (Trade Mark), was introduced which shows a significantly stronger performance effect in most detergent segments compared to other traditional commercial amylases. This enzyme has a broader pH and temperature range as compared to earlier amylases, meaning that in a typical detergent, for example, nearly equal performance is obtained at both 30°C as at 40°C. Furthermore, the washing time can also be reduced but still nearly, the same wash performance will be reached (Roy and Mukherjee 2013).
α-Amylase in food industry

α-amylases have been approved and used for many years in food manufacture. Approval of the use of this enzyme has advantages for food manufacturers by providing a different source of the α-amylase enzyme. The first, which has greater thermal stability and produces a different sugar profile. There are no significant disadvantages to food manufacturers, consumers or government agencies. In 2003 the German food industry employed around 525,000 workers in approximately 5,880 businesses and had a total turnover of around € 128 billion. However, similar to other branches of German industry, it faces increasing international competition. Innovations could be the key to new product areas. Therefore, experts take an optimistic view of growth in the area of functional food (Sá FC, Vasconcellos RS et al. 2013). The world market volume for functional food could rise to about US$ 10 to 22 billion. Future prognoses anticipate annual growth rates in the world market in excess of 20 %, one reason for the increasing demand could be that many food products today, such as chocolate pudding, ketchup, spaghetti sauce, chilli sauce, fruit puree and baby food, contain starch in different forms (modified starches) in order to get, among other things, appropriate viscosity (Flaschel E, Bott M et al. 2006). In food production, enzymes have a number of advantages:

- They are welcomed as alternatives to traditional chemical-based technology, and can replace synthetic chemicals in many processes. This can allow real advances in the environmental performance of production processes, through lower energy consumption and biodegradability
- They are more specific in their action than synthetic chemicals. Processes, which use enzymes, therefore have fewer side reactions and waste by-products, giving higher quality products and reducing the likelihood of pollution. They allow some processes to be carried out which would otherwise be impossible. An example is the production of clear apple juice concentrate, which relies on the use of the enzyme, pectinase (Marrelli M, Loizzo MR et al. 2013).

Therapeutical and Diagnostic application of α-amylase

α-amylase is widely used in pharmaceutical industry in various digestive aid preparations. Due to presence of bacterial α-amylase, starch in the consumed food is better digested and increase overall digestibility of food. Such digestive aid preparation is used for treatment of patient whose digestive power is reduced due to certain illness. Many such commercial
formulations of digestive aids are seen in drug stores either as syrup or as tablet (Faulks and Bailey 1990). The first clinical gene therapy is underway to correct an enzyme deficiency called Adinoseine deaminase (ADA) in children. There is a correlation between salivary α-amylase activity and pain scale in patients with chronic pain. The visual analogue scale (VAS) is commonly used to assess pain intensity. However, the VAS is of limited value if patients fail to reliably report. Objective assessments are therefore clearly preferable. Previous reports suggest that elevated salivary α-amylase may reflect increased physical stress. There is a close association between salivary α-amylase and plasma norepinephrine under stressful physical conditions. In this study, we have determined the usefulness of a portable salivary α-amylase analyzer as an objective biomarker of stress (Subramani and Aalbersberg 2012).

Enzymes are found in all tissues and fluids of the body. Intracellular enzymes catalyze the reactions of metabolic pathways. Plasma membrane enzymes regulate catalysis within cells in response to extra cellular signals, and enzymes of the circulatory system are responsible for regulating the clotting of blood. Almost every significant life process is dependent on enzyme activity. α-amylase production in aqueous two phase systems by another species of bacteria Bacillus in polyethylene glycol (PEG 10 000)/dextran (505 000) aqueous two-phase systems at various concentrations. An increase in the PEG concentration from 7 to 25 % (w/w) in the aqueous two-phase system resulted in an increase in the phase volume ratio with a concomitant decrease in the partition coefficient (K) and recovery of α-amylase in the top phase. However, varying dextran concentrations from 2.5 to 10% (w/w) decreased both the phase volume ratio and the partition coefficient of α-amylase at dextran concentrations lower than 2.5% (w/w) the phases could not separate (AKCAN 2011). Bacillus species have traditionally been utilised in the production of industrially important enzymes for a number of reasons: with the exception of the Bacillus cereus group, they are all generally recognized as safe (GRAS) listed and they are widely distributed in nature, they are easily cultured in relatively inexpensive media, and members of the genera Bacillus are capable of producing large quantities of many desirable enzymatic activities, most of which are secreted extracellularly into the fermentation medium. Extra cellular production of enzymes obviously simplifies subsequent product recovery and purification. Enzyme therapy is an effective safe alternative way in cancer treatment by reversing the tumour from malignant to benign. This could be applied, through its function. Firstly, by restore the body internal environment; this includes blood pH to be slightly alkaline, eliminate body waste, also restore intestinal
bacteria balance, strengthen the immune system, improve digestion and facilitate execration. Secondly, enzyme has decomposition effect through breaking down the fibrin coating of the cancer cell to unmask it in order for the immune system to identify as (non-self) and attack and destroy it. Thirdly, throughout blood purification by eliminate wastes decompose toxin; maintain right blood pH and fluidity; facilitated blood circulation when blood circulate well so it can transport oxygen and nutrient to cell and cancer cell can not live in oxygen rich environment. The enzyme therapy has a valuable advantage on conventional treatment; that enzyme therapy neither treats the underlying cause and the whole body nor the symptom, therefore it is considered a natural, non toxic, no side effects and easy to use.

CONCLUSION
Enzymes are tools of nature using natures’ own technology in modern industry associated with many advantages, compared to chemicals. Amylases have a great commercial value in biotechnological applications ranging from textile, bio washing, food, therapeutic and pharmaceutical industries. These uses have placed greater stress on increasing amylase production and search for more efficient processes.

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REFERENCES


