enzymeworld

NOV-DEC '07

brewing success with enzymes

contents

The Editorial Team

Compiled By:
Mr. Piyush Rathi
Mr. Dipak Roda
Ms. Ritu Pance
Mr. Mike Smith

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022-2583 8350/54
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advanced enzymes
Where enzyme is life

Dear Friends,

Every issue of Enzyme World has been increasingly appreciated by its readers, and this has prompted our Editorial Team to do better! Many thanks for your encouragement for their effort, and for the creation of a DIVINE SOCIETY. His Holiness Gurudev Pooiya Sri Sri Ravishankar guides us all to create this whole world as a Divine Society, where we may all live in harmony without causing damage to any living entity in the entire cosmos! This is a true celebration of life.

As India is striving towards 9%+ GDP growth rate, energy in all form has become quite an important issue along with infrastructure for sustainable and all-inclusive growth. Bio-ethanol and Bio-diesel are key initiatives in this segment. Hence, being India's largest Enzyme Company, we have taken it upon ourselves to make all Indians proud.

This issue of Enzyme World features some facets of this very interesting technology. Cane Sugar Molasses based Alcohol is the main stay for Ethanol production in India. With our ALCOZYMOST (a scientific mixture of enzymes, bio-nutrients, and anti-microbials) at a ppm dosage we have seen that we easily improve the output by 7%. This alone will lead to a whopping increase of 240 Million Litres of extra alcohol worth approximately Rs. 480 crores to economy.

While our SFSoil DG (our Enzymatic Oil Degumming Solution) will make the wholly chemical based Vegetable Oil Refining ECO-SAFE. It will also lead to significant saving of oil loss that occurs in the chemical processes. De-gummed oil is also very important for the production of Bio-diesel.

This brings us to another growing industry - beer production, where barley malt, rice and other grains are used to produce beer. Brewing is an art as the Brew Master deals with ever changing natural raw material, yet consumers expect constant quality, flavour and colour from their favourite brew! At Advanced Enzymes, we can certainly help make brewing an exact science, and make the Brew Master's job easier. To address this we offer a complete range of products such as SEBMull Plus, Progarazyme, Starzyme HT120L, Dextro 300L, Garazyme and SEBMature L.

Enzymes play a very vital role of maintaining 'immunity' in our body. Today, we offer 'immunity enhancing' enzymes with our world famous product IMMUNOGEB. This scientific blend of enzymes has been developed at the state-of the-art R&D centre of our parent company Specialty Enzymes in California, USA, under the expert guidance of one of the world's leading enzymologist - Mr. Vic Rathi. All of these enzymes are naturally occurring. We have only carefully extracted them, preserved and presented them for use as and when you need them most.

Several other developments are proceeding at this seemingly small biotech company. Our team of over 70 dedicated scientists along with over 25 other R&D organisations, universities and companies have been constantly pursuing side-effect free and ECO-SAFE solutions.

Another inspirational development: our production team under the able guidance of Biochemical Engineer Mr. Mukund Kabra and Microbiologist Dr. Anil Gupta, have achieved a rare feat. They have made our Sinner Plant (Near Nasik, Maharashtra) the only ZERO-DISCHARGE Enzyme Manufacturing Plant in the world! This plant has been functioning in this capacity for the last 3 months.

Gurudev Poojya Sri Sri Ravishankarji said, "We must first take responsibility for problems and practices ourselves, before we can request any other". His golden words have truly helped everyone in Advanced Enzymes take this step. We very well knew that His blessings are with us. It did involve a huge investment and very high treatment costs leading to erosion of the company's profits. Yet we all at Advanced Enzymes are truly proud to have taken this bold step. This achievement is now being lauded by all our colleagues in the Enzyme and Biotechnology industry.

We look forward to offer our knowledge and services to anyone and everyone in making this world a more beautiful place to live in; not just for us but for the many generations to come!

Jai Gurudev

Chandrakant Rathi

A great brew made easy!

Enzyme Solutions for the Brewing Industry

Brewing beer is really a very simple process, a unique mix of art and science that consists of a number of key steps. Brewing begins with malted barley that is milled and mixed with hot water to form a 'mash'. During mashing, the malt starches are converted to sugars. The sugar rich water is then strained through the bottom of the mash and is now called wort. The wort then goes to the brew kettle where it is brought to a boil. During this stage, hops are added at different times during the boil for either bitterness or aroma. The wort is then cooled and aerated, and brewers' yeast is added for fermentation. The yeast produces alcohol and carbon dioxide and other byproducts from the sweet wort. After fermentation the 'green beer' undergoes maturation. The last step in the brewing process is filtration, and then carbonation. Next the beer is moved to a holding tank where it stays until it is bottled or kegged.

Mashing

Mashing is the process of mixing milled grain with water, and heating this mixture up with rests at certain temperatures to allow enzymes in the malt to break down the starch in the grain into sugars, typically maltose.

Issues:

- Poor/low quality malt
- Adjuncts
- High Mash Viscosity
- Low Amino Nitrogen
- Low Saccharification

Lautering

Separating of the extracts won during mashing from the spent grain to create wort. Achieved in either a lauter tun or a mash filter.

Lautering has two stages:

- Wort run-off
- Sparging

Issues involved:

- High temperatures > 74oC
- Solubilizing maximum starch and fermentable sugar
- Fouling or slow filtration

Boiling

Intense boil for 60-120 min depending on its intensity, the hop addition schedule, and volume of wort the brewer expects to evaporate. Ensure wort sterility. Hops are

added, for bitterness, aroma and flavour to the beer. Proteins in the wort coagulate and the pH of the wort falls.

Fermenting

Yeast is added to cooled wort. Sugars won from the malt are metabolised into alcohol and CO2. Product is now called beer. Fermentation is carried out in Cylindroconical Vessels (CCVs) or Open Fermentation Vessels, made of stainless steel or wood.

Issues involved:

- Diacetyl formation
- Drop in pH may inhibit fermentation and affect

Taste and yield:

- Filtration issues

Conditioning

Beer is cooled to around freezing; proteins coagulate and settle out with the yeast. Unpleasant flavours such as phenolic compounds become insoluble in the cold beer, and the beer's flavour becomes smoother.

Conditioning issues:

- Cold temperature haze
- Carbohydrate complexes with ß-Glucan, polyphenols and polypeptides
- Diacetyl content

Filtering

Filtering the beer stabilises the flavour, and gives beer its polished shine and brilliance.

Rough filtration: Leads to cloudiness in the beer, but it is clearer than unfiltered beer.

Fine filtration: Beer with no noticeable cloudiness.

Sterile filtration: Almost all micro-organisms in the beer are removed.

Issues involved:

- Filtration rate
- Filter life

Filling & Packaging

Packaging is putting the beer into the containers in which it will leave the brewery in labelled bottles, kegs and casks, or bulk tanks for high-volume customers.

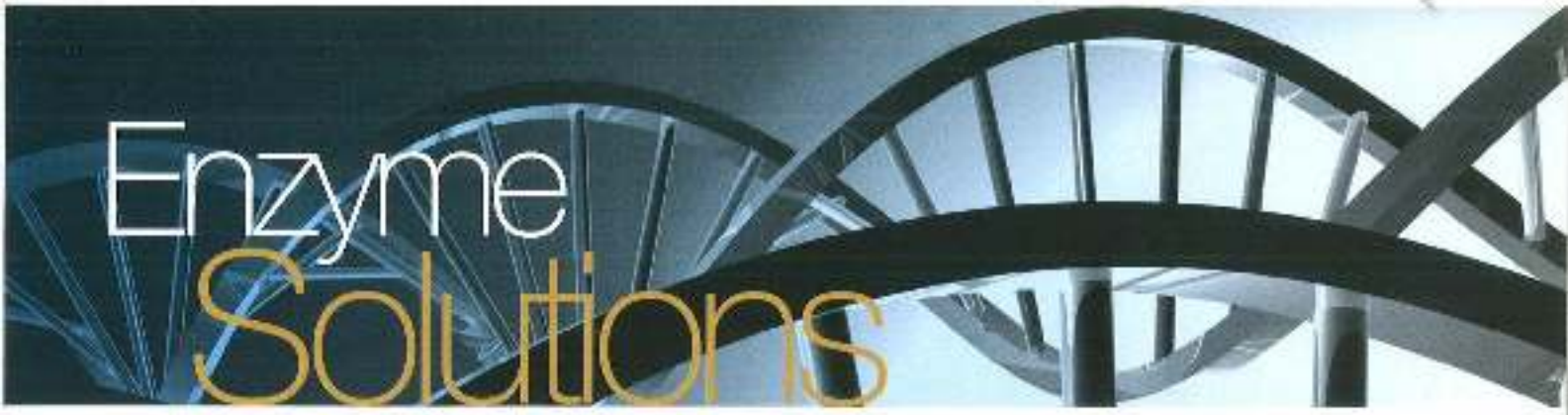
- Low calorie beer
- Applied to mash or during fermentation
- Optimal temperature range 50-60°C
- Optimal pH range 3.5-4.5

SEBmature L

- Alpha-acetolactate decarboxylase (ADLC) of fungal Origin
- It prevents formation of diacetyl by catalysing The decarboxylation of alpha-acetolactate to acetoin
- Maturation period can be eliminated or greatly reduced
- No or reduced di-acetyl rest period
- Applec to pitched wort before fermentation
- Optimal temperature range 0-20oC
- Optimal pH range 4.0-6.0

Depending on the composition of the grist and the adjuncts used, the brewer uses Proganozyme, SEbmalt Plus and Clarazyme at Mashing & Lautering stage. Clarazyme is also used for chill proofing of beer. Tanzyme HT 120L is excellent when high gelatisation • Temp adjuncts like rice is used. Dextro 300L is extensively used for low calorie beer during fermentation and SEBmature L is used during maturation to reduce maturation time.

- Mr Surendra Rao,



Enzyme Solutions

Proganozyme

- Fungal Beta-glucanase obtained from Trichoderma reesae
- Break down Beta-glucans
- Increased extract yield up to 20%
- Lautering time is reduced
- No cofactor (Ca) requirement
- Improved filtration
- Optimal temperature range 30-70°C
- Optimal pH range 4.0-7.5

SEBmalt Plus

- Balanced combination of enzymes viz protease, alpha-amylase and beta-glucanase from bacillus subtillis SP
- Satisfactory extract yield
- Improved wort run-off and beer filtration
- Increased free alpha amino nitrogen in wort
- Prevention of glucan and starch hazes

Clarazyme

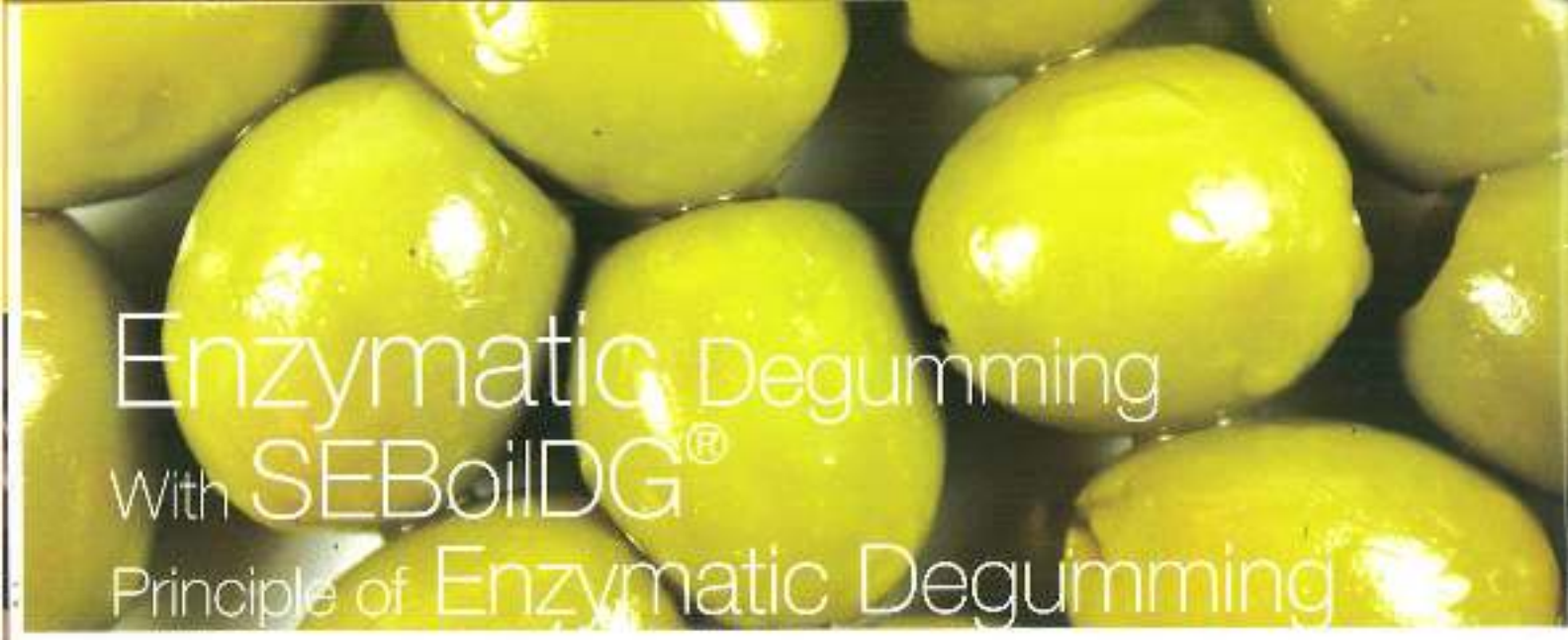
- Combination product of protease and Beta-glucans
- Prevents haze due to carbohydrate or proteins in the beer
- Stabilise, clarify, chill-proofing of beer
- Extended shelf life of final beer
- Ideal when adjuncts are used
- Optimal temperature 37°C
- Optimal pH range 4.5-5.0

Starxyme HT 120L

- Alpha amylase from Bacillus
- Liquify starch at Lautering temperature
- Increased wort yield with/without adjuncts
- Reduce mash viscosity
- Highly efficient with grain adjuncts
- Boiling of malt can be avoided
- Capable of replacing all of the malt for adjunct
- Optimal temperature range 75-95°C
- Optimal pH range 5.8-7.0

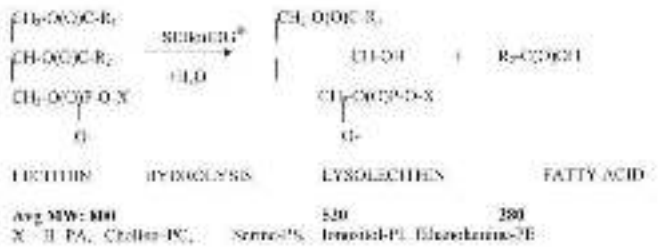
Dextro 300L

- Gluco-amylase obtained from Aspergillus Niger
- Liberate glucose units from the non-reducing ends of starchy polymers
- Reduce starch haze
- Reduced wort viscosity



Enzymatic Degumming With SEBoilDG®

Principle of Enzymatic Degumming



Degumming

PA is completely non-hydratable, PE is partially non hydratable and PC, PS & PI are completely hydratable. All types of Lysolecithin are completely hydratable.

SEBoilDG® is phospholipase A2 and hence it works by hydrolyzing the ester bond at C2 position of all types of phospholipid (gum) molecules. As a result, a hydroxy group gets added at C2 position and one molecule of free fatty acid is generated from every molecule of phospholipid (gum) hydrolyzed. The hydroxyl group generated at C2 position makes the phospholipid molecule extremely hydrophilic in nature. After hydrolysis with SEBoilDG® the hydrophilic lyso phospholipids move from oil phase in to water phase. This water is separated from the oil using a centrifuge and all the lyso phospholipids come out of oil together with the water. SEBoilDG® effectively reduces the phosphorous levels in degummed oils.

Vegetable Oil Processing Using SEBoilDG®

Vegetable oil is an important source of human nutrition. One gm of oil contributes as much as 9 calories of energy as against an energy contribution of 4 cal per gm of carbohydrates. Vegetable oils are essential for human nutrition as they are an important source of essential fatty acids and precursors of essential fatty acids. Vegetable oils supply fatty acids which get converted in to various compounds in the body that are essential for maintaining several key functions. Deficiency of essential fatty acids can lead to serious disorders. The recommended daily intake of oils & fats is about 60 gm per day for an adult.

Manufacture of Refined Vegetable Oils

Manufacture of vegetable oils involves two stages. The first stage is extraction from oil-bearing materials and the second stage is refining to remove odours as well as those components that could cause problems for stability of vegetable oils during subsequent storage.

Extraction of Vegetable Oils

Vegetable oils are produced on a commercial scale either through expelling of oil seeds in mechanical expellers or through the use of organic solvents known as solvent extraction. Typically, this expelling process is used for those oil seeds which have an oil content of more than 30-35% and solvent extraction is used for those oil seeds or oil-bearing materials which have an oil content of around 20% or less.

Refining of Vegetable Oils

Refining of vegetable oils include removal of gums or phospholipids which is known as degumming, colouring matter, oxidative materials, heavy metals, waxes, free fatty acids and odours from vegetable oil. The most commonly practiced method for refining of a majority of vegetable oils is called alkali refining or chemical refining. The other less commonly employed route is physical refining. Physical refining is currently being practiced for refining of palm oil.

Chemical Refining

Chemical refining involves use of caustic solution to neutralise free fatty acids present in the oil and convert them into soap. This soap is separated from the oil using a centrifuge. This is followed by washing with hot water to remove residual soap, bleaching using clay to remove colouring matter, oxidative material, metals and some other impurities, and finally the oil is deodorised.

Advantage of Chemical Refining

The advantage of chemical refining is that the neutralisation step also removes gums, heavy metals, a lot of colouring matter, some of the other impurities and also makes the remaining colour susceptible to bleaching. Therefore, usually chemical refining produces oil with excellent storage stability.

Drawback of Chemical Refining

i) The main draw back of chemical refining is that the soap generated by neutralisation of fatty acids with caustic, takes away a lot of neutral oil together with it when soap is separated from oil. The total loss of neutral oil, in general, could be anywhere between 0.25 to 2 times the weight of fatty acids depending upon various factors.

ii) In addition, a part of the soap generated during neutralisation does not get separated in the centrifuge. It gets carried forward in the oil. In order to remove this soap, oil is subjected to water wash.

This water wash takes away some amount of neutral oil together with soap, resulting in oil loss as well as generation of effluent.

iii) As mentioned before, the soap generates in the neutralisation step carries lot of neutral oil as well as gums. The neutral oil and fatty acids are recovered from soap by splitting the soap with sulfuric acid under hot conditions and removing the sodium sulfate as well as residual sulfuric acid by washing with large quantities of water. After removing the acid and salt, the gums are allowed to settle and the upper layer of fatty acids and neutral oil are separated through decantation. This process is known as acid oil generation. This step too generates a lot of effluent, causes loss of neutral oil, involves heavy consumption of chemicals, uses a lot of energy and in general is a messy operation. Also the mixture of fatty acids and oil recovered in this process is of low quality and gets sold at much lower price.

All the above drawbacks of alkali refining are directly related to the level of free fatty acids present in the crude oil and to some extent to the level of gums present in the crude oil. The higher the levels of free fatty acids, the higher are the losses.

Need for an Alternative Refining Process

These losses need to be eliminated especially at a time when the demand for vegetable oils is

steeply increasing, thanks to the diversion of part of the vegetable oil produced in the world for production of bio-diesel. This prevention of loss assumes all the more importance in a country like India, which is one of the largest importers of vegetable oil in the world. India

in the world for production of bio-diesel. This prevention of loss assumes all the more importance in a country like India, which is one of the largest importers of vegetable oil in the world. India produces about 7.5 million ton of oil per annum and imports about 5 million ton per annum, which is 40% of its requirement. The way to prevent these refining losses is to adopt physical refining. Since the losses in chemical refining are related to the level of free fatty acids and gums, physical refining results in significant savings in refining of vegetable oils with higher levels of free fatty acids and gums.

Physical Refining

Physical refining refers to the removal of free fatty acids from oil using a physical process through steam distillation under vacuum. However, distillation to remove fatty acids is the last step in the physical refining process. The first step is removal of gums or phospholipids, which is known as degumming. This is followed by bleaching with clay to remove colouring matter, oxidative material and some metals, and then the oil is subjected to vacuum distillation to remove free fatty acids. In order to produce refined oils with good storage stability through physical refining, the essential requirement is that the oil should contain less than 5 ppm of phoschorous before it goes to deodoriser. Hence the degumming process has to be very efficient. Though there are several degumming techniques known to the industry today, Enzymatic Degumming is the best alternative that produces consistent degumming results with very low levels of residual phosphorous in degummed oil. In addition, enzymatic degumming causes the least amount of neutral oil losses in degumming. SEBoilDG® gives excellent degumming results on all types of vegetable oils at temperatures as low as 35°C.

- Dr. Rao

ImmunoSEB®

An Enzymatic Approach to Enhanced Immune Function

ImmunoSEB® is a unique blend of enzymes and lactoferrin that not only supports the immune system, but also actively kill pathogenic bacteria. ImmunoSEB® contains six major components: lysozyme, catalase, lactoperoxidase, Peptizyme SP®, bromelain and lactoferrin. These are all naturally occurring compounds that act synergistically to support and defend the body against potentially harmful pathogens.

Lactoferrin is an iron binding glycoprotein that is present in milk and other secretory fluids like tears, saliva, pancreatic juice, among others. As a supplement, it appears to have antibacterial, antiviral, antifungal, anti-inflammatory, antioxidant and immunomodulatory activities. Lactoferrin demonstrates bacteriostatic activity against a wide range of microorganisms including gram-negative bacteria like E. coli, Salmonella, and other coliforms. A possible explanation for its activity is its iron binding facility, which may inhibit bacterial growth through iron deprivation. 1-5

Lysozyme is a naturally occurring enzyme that attacks the cell wall of certain bacteria. This activity gradually weakens the cell wall until osmotic pressure inside bursts the structure, destroying the bacteria. Lysozyme in the human body acts as a protective barrier against environmental agents and, in doing so, helps prevent infections. Further, it acts synergistically with lactoferrin to potentiate the activity of both proteins. 6

Peptzyme SP is a serratopeptidase, one of the most potent anti-inflammatory enzymes available today. This anti-inflammatory activity reduces the inflammation associated with infection. 7

Catalase is a natural anti-oxidant, especially effective against peroxides. It also works in coordination with other systems to enhance immune activity. 8

Lactoperoxidase is a natural anti-oxidant enzyme, but has no direct anti-bacterial effect on its own. However, lactoperoxidase does work synergistically with lactoferrin, immunoglobulin and lysozyme, in the presence of the co-factors hydrogen peroxide and thiocyanate. The reaction that takes place produces compounds that are profoundly antibacterial and plays a key role in protecting mucus membranes against bacterial invasion. 8

Bromelain is well known for its anti-inflammatory properties.

The combination of ingredients in ImmunoSEB® provides a powerful approach to strengthening the body's immune system. Further, taken as a dietary supplement, ImmunoSEB® may not only strengthen the immune response, but also directly help protect the body from pathogenic organisms.

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- Mr. Mike Smith

Enzyme Quiz

1. Which chemical is classified as an enzyme?

(1.) galactose (2.) lipid (3.) protease (4.) manganese dioxide

2. Which element is present in maltase, but not in maltose?

(1.) carbon (2.) hydrogen (3.) oxygen (4.) nitrogen

3. In enzyme controlled reactions, the role of certain vitamins such as niacin is to act as

(1.) an enzyme (2.) a substrate (3.) a coenzyme (4.) a polypeptide

4. Salivary amylase is an enzyme in humans that breaks down starch. The optimum pH for this reaction is 6.7. The rate of this reaction would not be affected by

(1.) maintaining the pH of the reaction at 6.7 (2.) substrate concentration (3.) enzyme concentration

(4.) decreasing the temperature of the reaction by 5°C

5. A certain enzyme will hydrolyze egg white but not starch. Which statement best explains this observation?

(1.) Starch molecules are too large to be hydrolyzed. (2.) Enzyme molecules are specific in their actions.

(3.) Egg white acts as a coenzyme for hydrolysis.

(4.) Starch is composed of amino acids.

6. Lipase, maltase, and protease are members of a group of catalysts known as

(1.) hormones (2.) carbohydrates

(3.) lipids (4.) enzymes

7. Which molecule is not associated with the reaction that is occurring in the solution?

(1.) A (2.) B (3.) C (4.) D (5.) E

8. Which enzyme represents an enzyme functioning in this reaction?

(1.) A (2.) B (3.) C (4.) D (5.) E

9. Which substance most likely represents letter C in reaction two?

(1.) amylase (2.) protease (3.) sucrase (4.) lipase

10. A student placed groups of 50 seeds in a variety of temperatures ranging from 0 to 5°C. A difference in the rate of germination observed in the groups at different temperatures was most likely due to the effect of temperature on

(1.) ammonia (2.) acids (3.) enzymes (4.) cellulose

FEATURE
ENZYMES

CHANGE AGENTS

A big-ticket acquisition in the enzymes segment has domestic players discomfitted, but not enough to head for the door

DHRUV RATHI and NANDITA DATTA with SUDIPTO DEY

FOR A clutch of small, home-grown enzyme makers, Biocon's October sale of its enzymes business to Novozymes was an unsavoury moment. On the one hand, it liked their spunk—Biocon sold the Rs 95-crore business for Rs 167 crore. That was a fancy valuation. Very few companies get acquired at a sales valuation multiple of five.

On the other hand, Biocon's sell-out to the $1.26-billion world leader No zymes conveyed one message—en zymes were becoming a high invest ment, high-risk business valued only for companies with huge research spends; small companies would struggle. And this isn't an Indian phenom enon—on October 30, 2007, soon af ter concluding the Biocon acquisition in India, Novozymes struck a deal to acquire 70% in Phylon Bio, a company founded in Canada in 1990, to strengthen its position in microbial products for the agricultural sector.

Biocon's enzymes busi ness, though highly profit able—generating a 39% pro fore interest and tax on it rose—grew by just under 7% over the past two years against

"It (competitor Novozymes' takeover of Biocon's enzymes business) was a wake-up call for us"

FEATURE

ENZYMES

"Users have not fully explored the potential of new enzymes developed in the last few years"

novozymes

"Businesses are very reluctant to change. They feel enzymes are expensive compared to chemicals"

Specialty Enzymes and Biochemicals Co.

advanced enzymes
Where enzyme is life

SEB GROUP

Corporate Office: Advanced Enzyme Technologies Ltd., 6th Floor, "A" Wing, Sun Magnetica,
Accolade Galaxy Complex, L.I.C. Service Road, Eastern Express Highway, Louiswadi, Thane-400604, India.
Tel.: +91-22-2583 6300/6152/53/54, +91-22-2563 1872, +91-22-2562 0199
Fax: +91-22-2583 5169/6197 Email: info@enzymeindia.com Website: www.enzymeindia.com