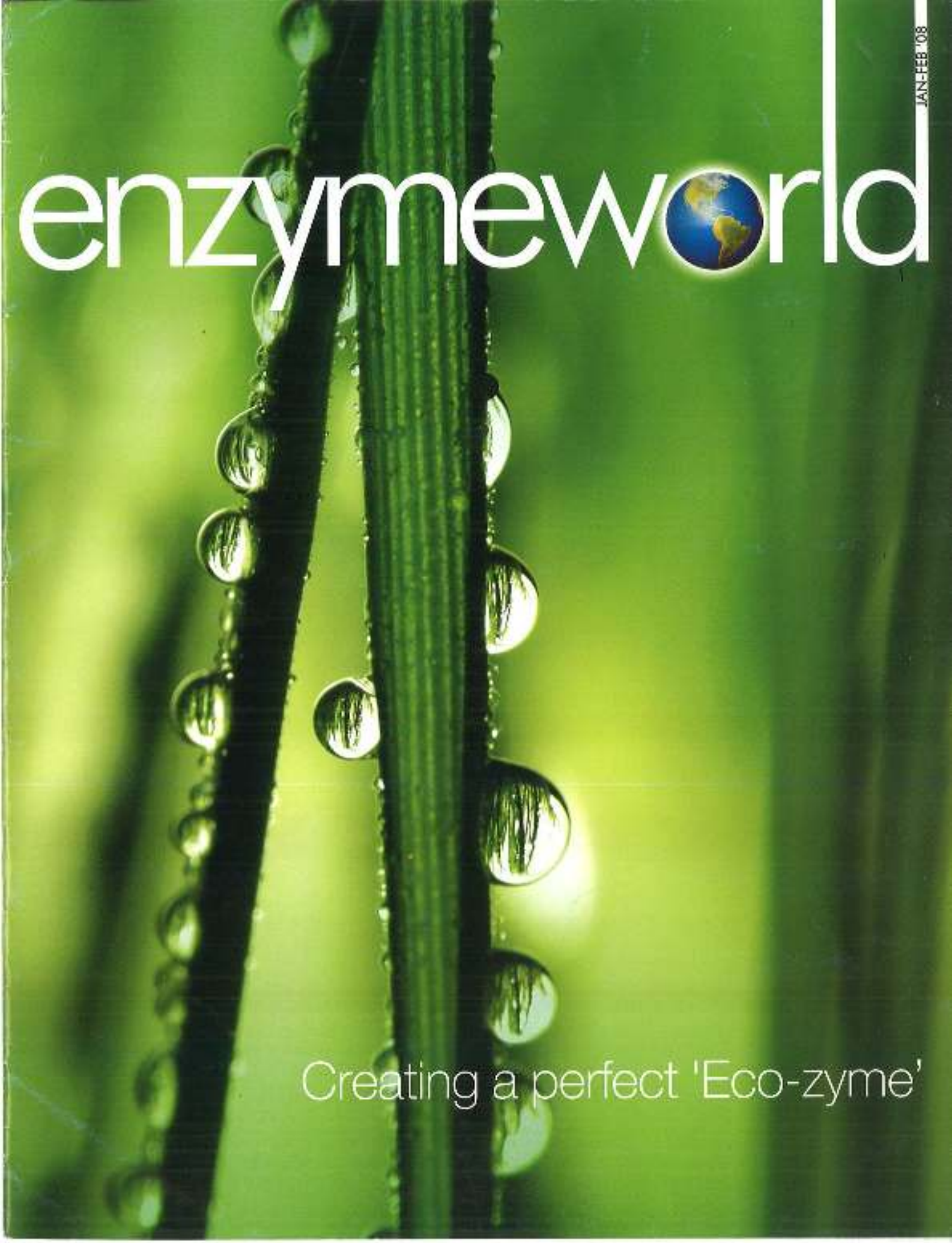
JAN-FEB '08

# enzymeworld



## Creating a perfect 'Eco-zyme'

# contents

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advanced enzymes
Where enzyme is life

Dear Friends,

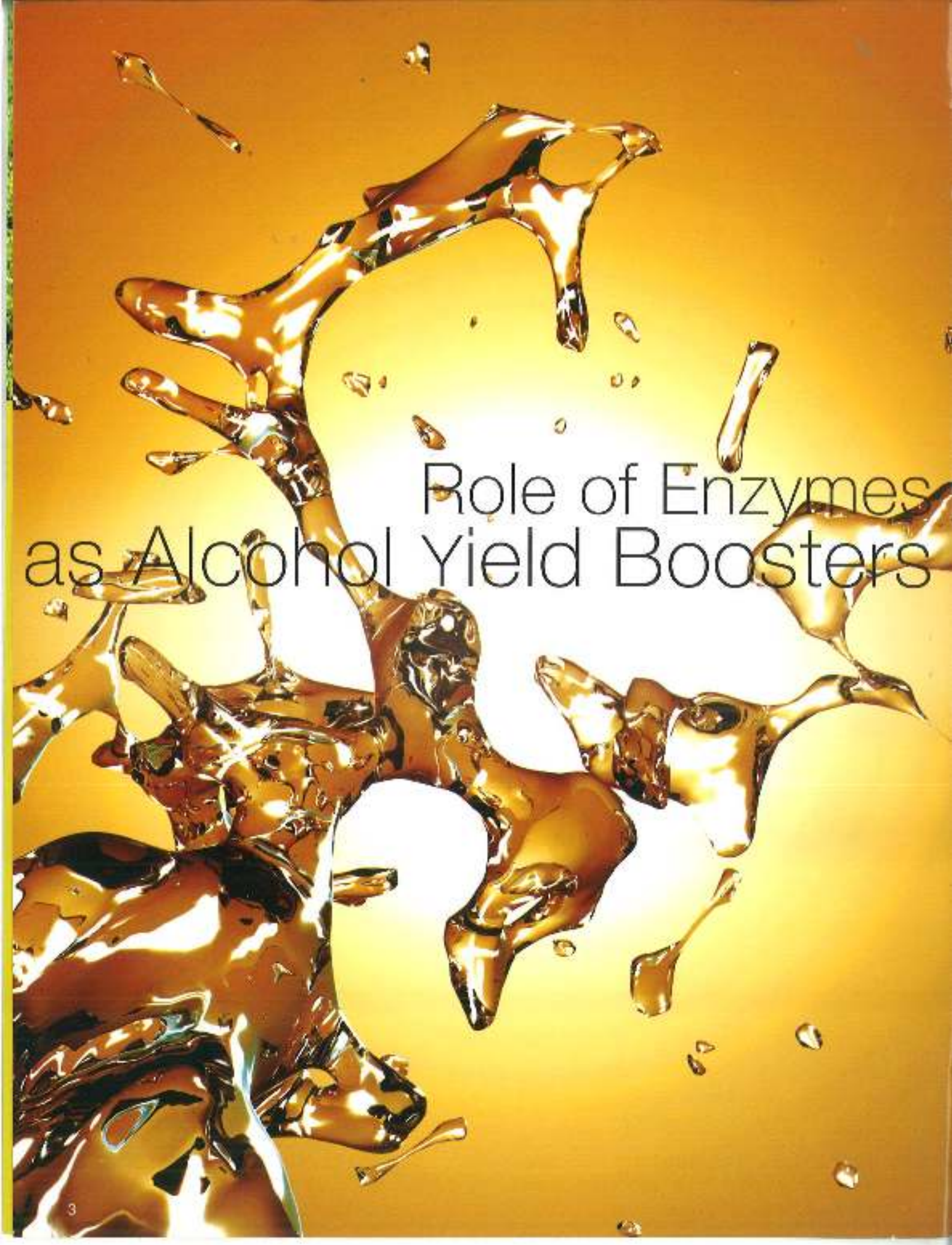
This issue of Enzyme World features some very interesting facts about the application of enzyme technology to cane molasses based Ethanol production. With Alcoboost, our proprietary technology, we have seen that the output improves impressively by 7% at a dosage of 5-10ppm. This alone leads to a whopping increase of 240 million litres of extra alcohol production, which is worth approximately $120 million to the Indian economy.

All grains contain phosphorous, bound up in phytate bonds and hence are not readily available. Use of the enzyme Phytase improves the availability of this phosphorus which in turn delivers better egg quality. As a natural consequence this also helps in reduction of the use of DCP in the feed formula and reduces phosphate pollution in the environment.

Enzymes are at the forefront in every activity related to "life". They are critical elements of our daily lives. We breathe, we eat, we laugh only due to the numerous enzyme systems working in our body. Yet the use of enzymes for disease prevention and treatment is almost restricted at the moment to a few segments such as digestive aids, anti-inflammatories and thrombolytics. We envisage a great future for the use of enzyme therapeutics in every possible disease indication!

Jai Gurudev.

C. L. Rathi,
Managing Director



# Role of Enzymes as Alcohol Yield Boosters

India imports nearly 70% (approximately 110 million tons) of its annual crude petroleum requirement. The prices of the crude oil ranges from US$ 60-70 per barrel and the expenditure on crude purchase is in the range of Rs.1600 billion per year; impacting the country's foreign exchange reserves in a big way. The robust growth in the Indian economy will further increase the energy requirements of India.

Taking into account the current scenario, the Government of India has recognized the need to develop alternate renewable sources of energy within the country and took several initiatives in this direction. One of the initiatives is blending of fuel ethanol with gasoline at 5% level in some select states in the first stage and then going to 10% level of blending from the last quarter of 2008. However, implementation of this program is dependent upon availability of sufficient quantity of ethanol in India at required price.

The estimated requirement of ethanol in India for blending with petrol at 5% level and at 10% level are as follows:

## Blending Level in Petrol

| | 5% | 10% |
|---|---|---|
| 2006-2007 (Million litres) | 573 | 1352 |
| 2016 -2017 (Million litres) | 1233 | 2526 |

Various sources are being considered by the industry for production of fuel ethanol in India. India, being the world's largest producer of sugar, has a production of about 11.5 MMT of molasses in the year 2006-'07. Molasses, therefore, becomes an obvious and natural choice of raw material for meeting the fuel ethanol requirements of India for the time being.

## Process

Ethanol is produced from molasses by fermentation with the help of yeast and recovering ethanol through distillation. The following reaction shows the chemical & mass balance of ethanol production from molasses.

$$C_{12}H_{22}O_{11} + H_2O \longrightarrow C_6H_{12}O_6 + C_6H_{12}O_6$$

Sucrose (342 gms) + Water (18 gms) → Invert Sugar: Glucose (180 gms) + Fructose (180 gms)

$$C_6H_{12}O_6 \longrightarrow 2C_2H_5OH + 2CO_2$$

Glucose (180 gms) → Ethyl Alcohol (2 x 46 gms) + Carbon dioxide (2 x 44 gms)

## Molasses Composition

| Components | Percentage |
|---|---|
| Fermentable Sugars (sucrose, glucose, fructose etc.) | 40% - 50% |
| Un-fermentable Sugars (raffinose, rhamnose, stychyose) | 5% - 7% |
| Other carbohydrates | 2 - 5 % |
| Ash | 7 - 15% |
| Vitamins | Varying amount |
| Nitrogenous Compounds (crude protein, amino acids) | 4.5 - 6% |
| Non-Nitrogenous Compounds (aconiticacid, citric mallic oxalic, polyphenols etc.) | 1.5 - 6% |
| Wax sterols and phosphotides | 0.1 - 1% |

## Molasses Fermentation

Molasses fermentation to ethanol is a complex set of reactions catalyzed by yeast Saccharomyces Cerevisiae. The initial stage of fermentation is carried out under aerobic conditions and the later stage is carried out under anaerobic conditions.

## Aerobic Stage

Aerobic stage is characterized by the growing of yeast cell culture in a molasses wort containing about 12 to 15 % sugar in a separate vessel called a pre-fermenter. Lots of care is taken in pre-fermenters to ensure proper growth of yeast and to create healthy yeast. Care is also exercised to arrest contamination. Several additives are normally added to get desired results in pre-fermenter.

Aerobic stage is usually carried out until a cell concentration of 2.5 - 3.0 x 108 per ml is reached in the wort. The concentration as well as health & vitality of yeast cells will have proportionate influence on fermentation time as well as yield of alcohol.

## Anaerobic Stage

The second stage starts after the required bub (yeast) is pitched in the ratio of 1:8 or 1:10 into the fermenter and fed with wort having required sugar concentration. Anaerobic stage is characterized by conversion of sugars present in molasses into alcohol. Yeast growth continues to take place during this stage.

Conversion of sugar into alcohol is an exothermic process and releases heat, causing rise in temperature. Hence, careful control of temperature within the optimum range for yeast fermentation is essential in order to get high fermentation efficiency. The recommended temperature range for efficient fermentation is 30-32°C.

It is also important to maintain proper pH of the wort to get high degree of fermentation efficiency. The recommended pH range is about 4.8 to 5.0. The pH of lower than 4.8 in the wort normally indicates high level of contamination by acid producing bacteria and it can normally coincide with high levels of total volatile acids in wort.

In the process of producing ethanol from molasses in order to get very high fermentation efficiency, in addition to maintaining right temperature, pH, yeast cell concentration and sugar concentration, it is also very important to control contamination, provide necessary nutrients and micro-nutrients needed for growth, health & vitality of yeast cells.

## Typical Problems Faced in Fermentation

- Stuck fermentation
- Increase in fermentation time
- Lower Alcohol yield

Contamination is the most common cause for all the above problems. This contamination is caused by the following:

- Primary and secondary infection in wort
- Abnormal relationship in composition of molasses and presence of volatile fatty acids. Infection of wild yeast and acid forming bacteria are greatly detrimental to fermentation.

The other factors that can cause some of the above-mentioned problems are:

- Insufficient concentration of yeast cells in wort
- Insufficient levels of nutrients as well as micro nutrients to maintain growth, vitality and health of yeast cells.

## Precautions to be Taken for Healthy Fermentation Conditions

Cleaning of all bulk vessels, pre-fermenters and fermenters must be done scrupulously and washed with dilute solution of bleaching powder or formalin and finally washed thoroughly with water. Other accessories like pumps, PHE and lines should also be washed thoroughly.

1. Air introduced in PF to be sterilized.
2. If the molasses by themselves are carrying a very high load of contamination, it is advisable to pasteurize molasses to reduce contamination.
3. If the contamination is resulting in drop in pH of wort, it is recommended to control pH through addition of suitable alkali.
4. It is highly recommended and almost essential to use antibiotics to control contamination and produce alcohol in good yield.
5. Ensure release of nutrients trapped in molasses, which could make significant contribution to enhancing the fermentation efficiency by providing the nutrients needed for yeast growth and vitality.

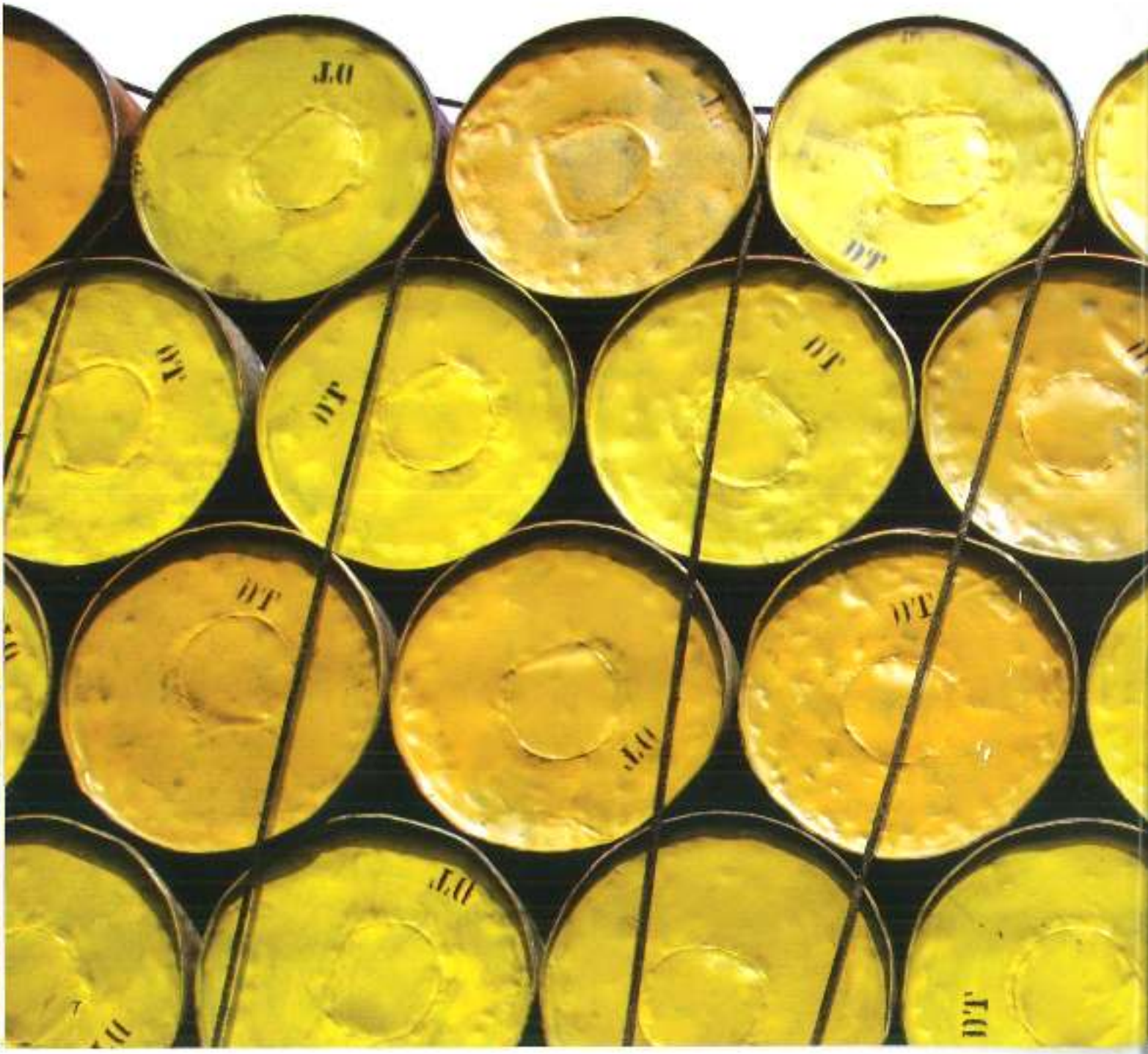
## Alco Boost- A Bio-nutrient for Healthy Yeast Growth

AlcoBoost is a complex blend of active agents that deliver strong antibiotic activity and provide the necessary nutrients for maintaining yeast growth, health and vitality of yeast cells. It is used as an alcohol yield booster in molasses fermentation plants and is 100% eco-friendly and safe for usage.

## Mode of Action

The antibiotics present in Alco Boost control contamination very effectively and the other active agents present in Alco Boost provide the necessary nutrients for maintaining growth, health and vitality of yeast cells. Absence of contamination results in conversion of the fermentable sugars into alcohol rather than to unwanted chemicals such as organic acids. This naturally increases the yield of alcohol.

Simultaneously, creating enough number of yeast cells in wort by providing yeast nutrients ensures completion of fermentation within the stipulated time. Elimination of contamination and availability of nutrients ensures health and vitality to yeast cells which result in higher fermentation efficiency.



## Boost Your Profit With Alco Boost

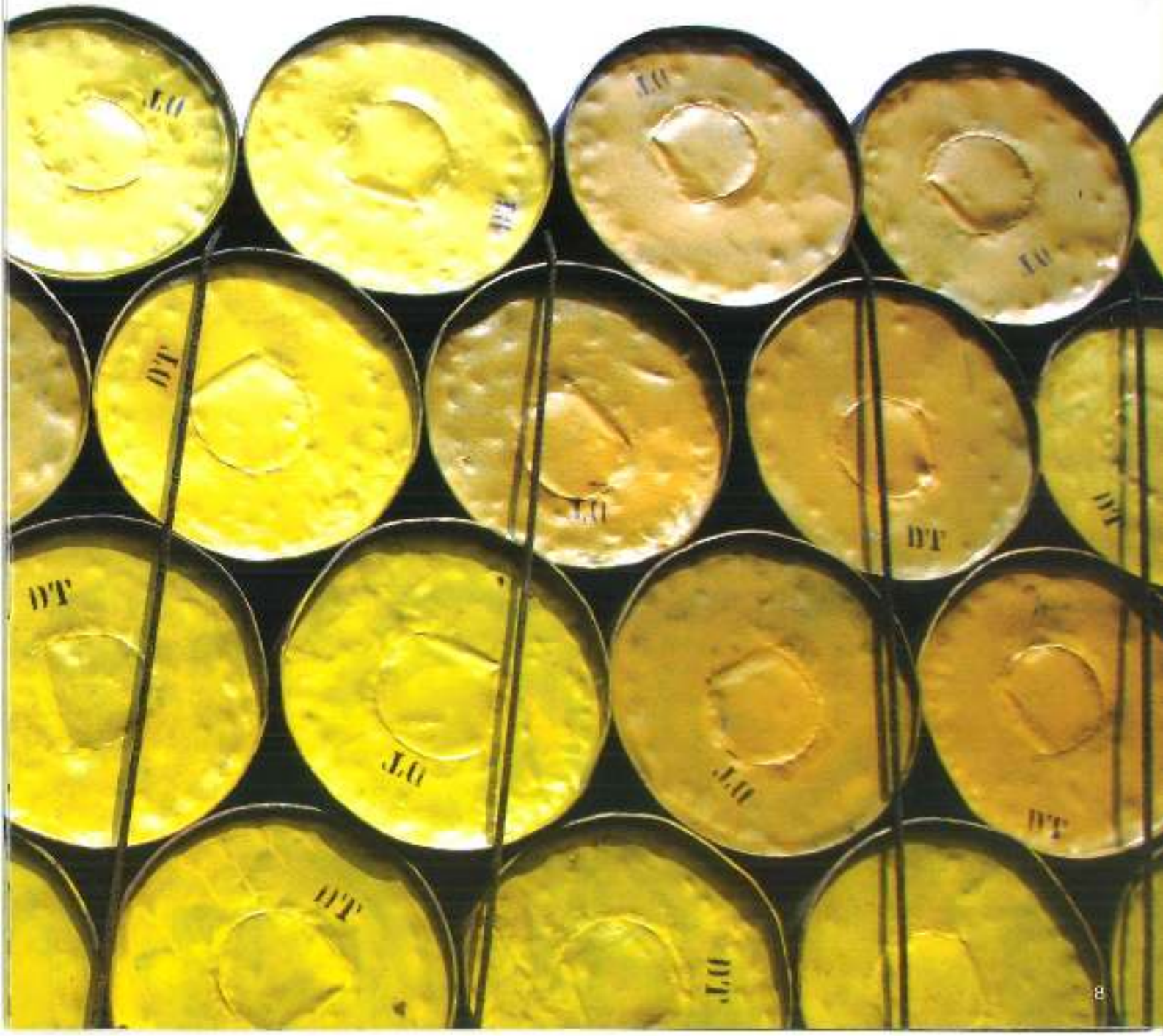
Use Alco Boost in molasses fermentation to get:

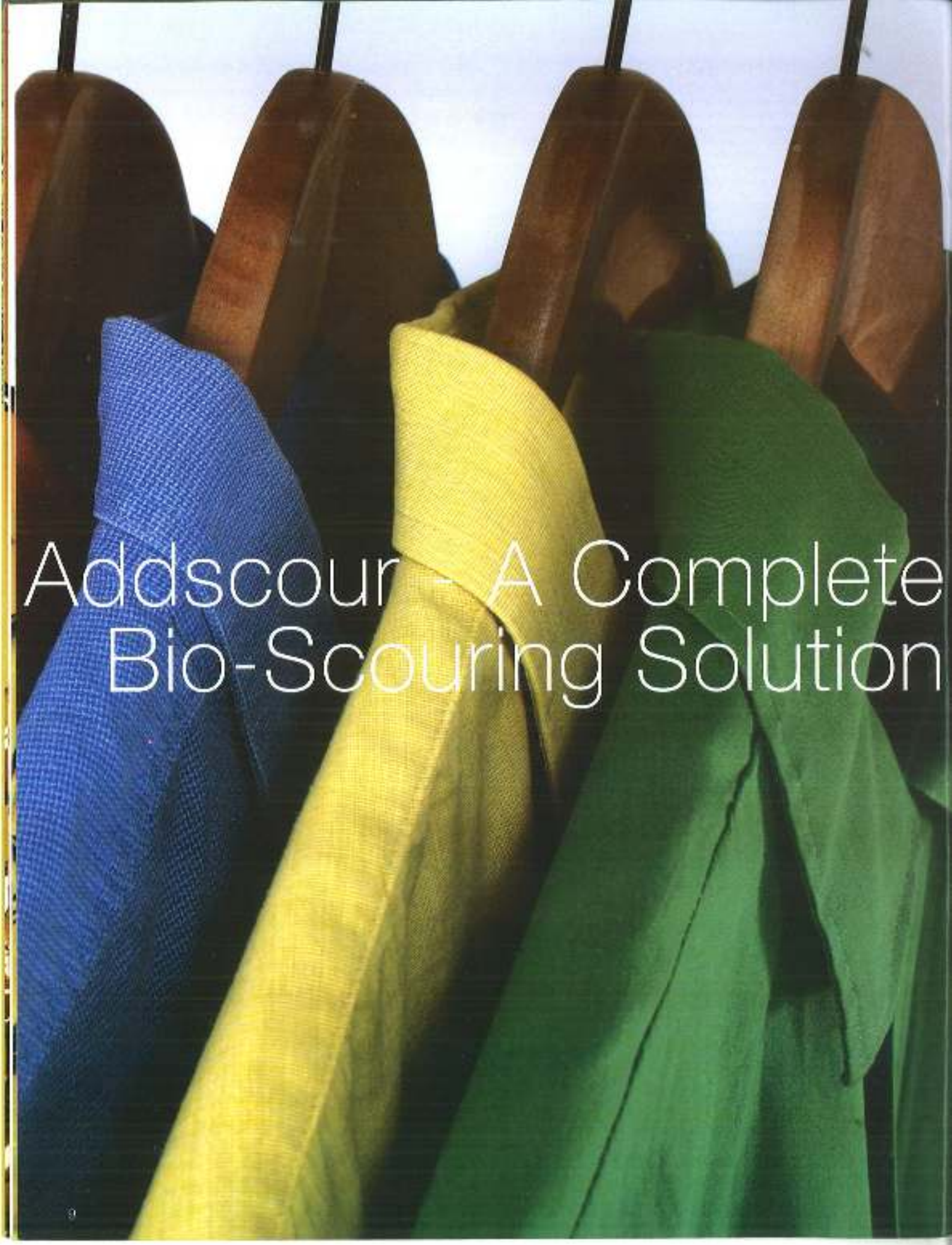
1. An increase in overall alcohol yield
2. Reduction in contamination
3. Reduced usage of urea and DAP
4. Significant reduction in retention time
5. Efficient & easier operation of the fermentation system
6. Increased profitability of distilleries

The above advantages are clearly observed when Alco Boost is used in 5-10 ppm level, depending on plant conditions. With good fermentation process in place, high fermentation efficiency is achieved.

Alco Boost has been well proven to take fermentation efficiencies of molasses distilleries beyond 90%.

- *Mr. Surendra Rao*




Addscour - A Complete
Bio-Scouring Solution

## Need of Bio-Scouring

Grey cotton or any form will be scoured in order to remove natural and foreign impurities and make the fabric Hydrophilic. Desired scouring can be achieved by removing wax and fat from Cuticle, Pectin, Hemicellulose and Proteinaceous matter from primary wall.

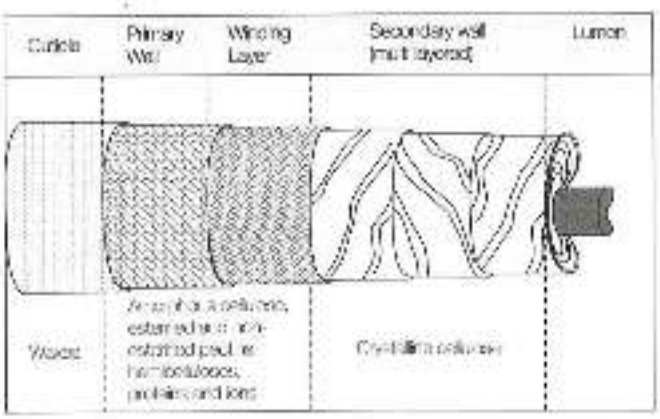
Today, highly alkaline chemicals like caustic soda, soda ash, silicate, and scaping agents are used for scouring to remove the non-natural impurities from the cotton. Use of 3-4% NaOH on weight of fabric, results in destruction of cotton structure. Intensive rinsing and more acid are needed for neutralization of cotton, which enlarges the volume of effluents. Furthermore, these hazardous chemicals result in increase in COD (Chemical Oxygen Demand), BOD (Biological Oxygen Demand) and TDS in the waste water. These chemicals also attack the cellulose leading to heavy strength loss and weight loss in the fabric. The net result is low quality cotton and polluted environment with high usage of energy, chemicals and water.

## Need of Bio-Scouring Package

In Bio-Scouring process, one can obtain better results using Bio-Scour Package. Enzymes which are substrate specific will only attack natural and foreign impurities resulting in better quality cotton and eco friendly environment.

## Cotton Structure

Cotton, when we studied in details, has the following structure:




Composition of dry matured cotton is as follows:

| Constituents | Composition [%] | |
|---|---|---|
| | Whole fibre | Outer layer |
| Cellulose/xylo-glucan | 94 | 54 |
| Waxes | 0.6 - 1.3 | 14 |
| Pectic substances | 0.9 - 1.2 | 9 |
| Protein/nitrogen substances | 0.6 - 1.3 | 5 |
| Ash | 1.2 | 3 |
| Organic acids | 0.8 | - |
| Others | 1.4 | 12 |

Here we can see that in outer layer, which include cuticle, primary and winding layer contains the impurities that we want to remove in order to get hydrophilic cotton. This specific attacking is possible only through enzymes that are substrate specific.

## AETL Bio-Scouring Package

Products

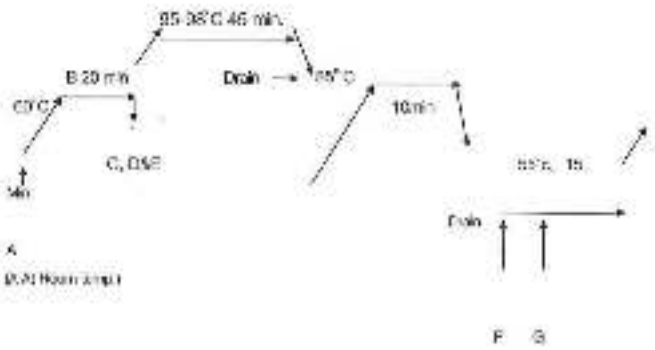
Addscour P: Blended enzymes for Bio-Scouring

Sebeux WDS: Dispersing agent

Sebwet CP: Wetting agent

## Bio-Scouring Process:

| | |
|---|---|
| Scouring & Bleaching | Hot wash |
| Neutralization & | Peroxide |
| Killing | |




Charge Sebwet CP-0.3% (A) at room temp, raise temp to 60 °C and add a dose Adcascour P-0.8B). Maintain temperature for 20 min, raise temp to 95 °C and charge Sebeux WDS -0.25%, $H_2O_2$ - $H_2O_2$ - 1.5% & Soda Ash - 1%.

## Results Analysis

A) Drop Test

| Method | Time (sec) |
|---|---|
| Alkaline scouring | Instant |
| Bio scouring | Instant |

B) Sink Test

| Method | Time (sec) |
|---|---|
| Alkaline scouring | 1.2 Sec |
| Bio scouring | Instant |

C) Weight Reduction

| Method | Percentage |
|---|---|
| Alkaline scouring | 7.89% |
| Bio scouring | 3.42% |

D) Whiteness

| Method | Whiteness Index |
|---|---|
| Alkaline scouring | 67.78 |
| Bio scouring | 67.23 |

E) Residual Bath

| Parameter | Plane water | Bio scoured Bath | Alkaline Bath |
|---|---|---|---|
| T.D.S | 200 ppm | 2300 ppm | <7000 ppm |
| Hardness | 55 ppm | 100 ppm | <250 ppm |
| pH | 7.4 | 10.0 | < 13 |

F) Pectin Content

| Residual Pectine (%) Ruthenium Red Test | |
|---|---|
| Alkaline scouring (Conventional) | 3850 |
| Bio Scouring | 1832 |

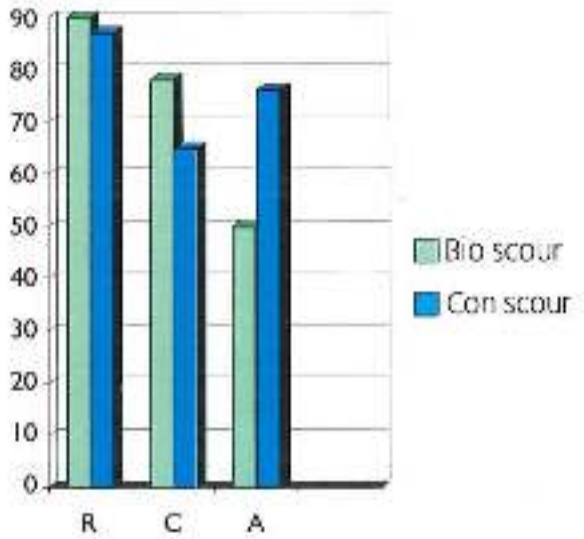
## BioScouring vs Conventional Scouring

G) COD

| COD values (mg 02/l) Residual bath | |
|---|---|
| Alkaline scouring (Conventional) | 3550 |
| Bio Scouring | 1632 |

H) Dye Exhaustion

- Reactive dye exhaustion increase by 4%
- Cationic increase by 20%
- Anionic reduce by 34%
- Pectic hydrolysis produces more negative charge on fabric




R- Reactive dye

C- Cationic dye

A Anionic dye

I) Finishing

| Application | Alkaline scouring | Bio scouring |
|---|---|---|
| Wicking height | 14.6 cm | 15.6 cm |
| Drop test | 1 sec | instant |
| Sink test | 2-3 Sec | instant |

J) Pilling

| Method | Pilling Scale |
|---|---|
| Alkaline scouring | 1-2 |
| Bio scouring | 2-3 |

## Conventional Scouring Process

| | |
|---|---|
| NaOH | -3% |
| Wetting agent | 0.5% |
| Sequestering agent | -0.5% |
| Lubricant | 0.3% |
| H2O2 | -1.5% |
| Stabilizer | -0.3% |
| Temp | -95OC |
| Time | -1hour. |

## Advantages

- Produces lower weight loss
- Less pollutant to the waste water, 60% reduction in COD, BOD and TDS
- Lower energy consumption
- Less aggressive for the fabric and for the environment
- Provides a safe working environment
- Softer cotton textiles
- Reduced water consumption
- Complete removal of non-cellulosic impurities
- Uniform and high absorptive pretreated goods
- High accessibility of cotton substrate for dyestuff uptake

- Dipak Roda

# Enzyme Therapeutics:
## India & Beyond

The quote by famous French writer Victor Hugo- "An invasion of armies can be resisted, but not an idea whose time has come"; would aptly describe the status of Enzyme Therapeutics today. It is the time to reintroduce to the world the potential of Enzyme Therapeutics. This paper will review the status of Enzyme Therapeutics in India and globally.

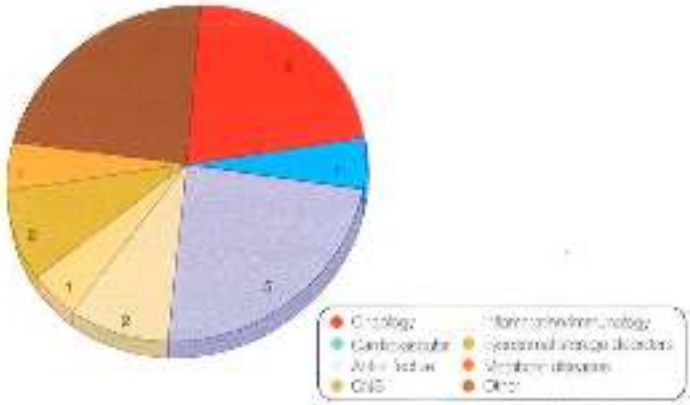
## Pharmaceutical Sector by 2020

The likely pharmaceutical sector scenario globally (1) will be a revolution of sorts for all concerned- global pharmaceutical companies, the healthcare decision makers and the patients. The issues outlined below will have a major bearing on all stakeholders:

- An aging population & a disease profile, changing from terminal to chronic, are to be seen.
- A shift in medical care decision making from doctors to patients; i.e. self-care and primary care providers. This translates into a shift from disease treatment to prevention. Ironically, majority of the current pharmaceutical spending is on disease treatment drugs.
- Medicines, particularly safe, efficacious and cost-effective; are being increasingly desired and sought. Findings of a new National Survey (2007) done in USA, finds a strong public interest in prescription drug safety issues, indicating that drug safety is a real concern. Unfortunately almost all drugs today have unwanted side effects.
- The decline in R&D productivity in many discovery programs of all the major pharmaceutical companies have been reported.

The solution undoubtedly is innovation, innovation in delivery systems as well as introducing novel innovative drugs. A trend is being observed towards this. Pharmaceutical companies have started moving towards niche products rather than blockbusters to improve productivity. The pie chart (Fig 1) shows 2008 drug approvals for New Molecular Entities (NMEs) and New Biological Entities (NBEs) by therapeutic area (2). Approvals of NMEs for Oncology are in the limelight followed by anti-infectives. Companies are moving away from developing innovative but risky therapies for large competitive markets in favour of developing drugs for smaller populations. This could be the end of the blockbuster, in favour of the 'nichebuster'. But more needs to be done in terms of making safe, efficacious, affordable and preventive drugs. Enzyme Therapeutics could be an effective solution in this new era of Biologics in modern medicine.

Therapeutic enzymes are a part of this group and also known as Biological Response Modifiers or BRMs.




Source: Various | Drug Discovery

## Biological Response Modifiers (BRMs)

BRMs are substances of biological origin such as antibodies, cytokines and other immune system substances that can be produced in the laboratory for use in disease treatment. These molecules, as described below, assist, stimulate and restore the body's ability to fight disease by duplicating action of internal cellular mediators.

- Monoclonal antibodies may be used to enhance a patient's immune response or as a drug delivery system.
- Interleukins are used to stimulate Natural Killer cells, T cells, and Macrophages; boosting the immune system's function.
- Interleukins such as IL2 stimulate the growth and activity of many immune cells, such as lymphocytes.
- Colony stimulating factors or hematopoietic growth factors encourage bone marrow and stem cells to divide and develop into white blood cells, platelets, and red blood cells.
- Vaccines - scientists are investigating ways that cancer vaccines can be used in combination with other BRMs.
- Gene Therapy - an experimental treatment that involves introducing genes into a person's cells to fight disease. Researchers are studying gene therapy methods that can improve a patient's immune response to cancer.
- Non-specific immunomodulating agents stimulate or indirectly augment the immune system. Often, these agents target key immune system cells and cause secondary responses such as increased production of cytokines and immunoglobulins.

Therapeutic Enzymes are also classified as important BRMs because they work together with the internal enzymes, interact and stimulate the immune system cells (7).

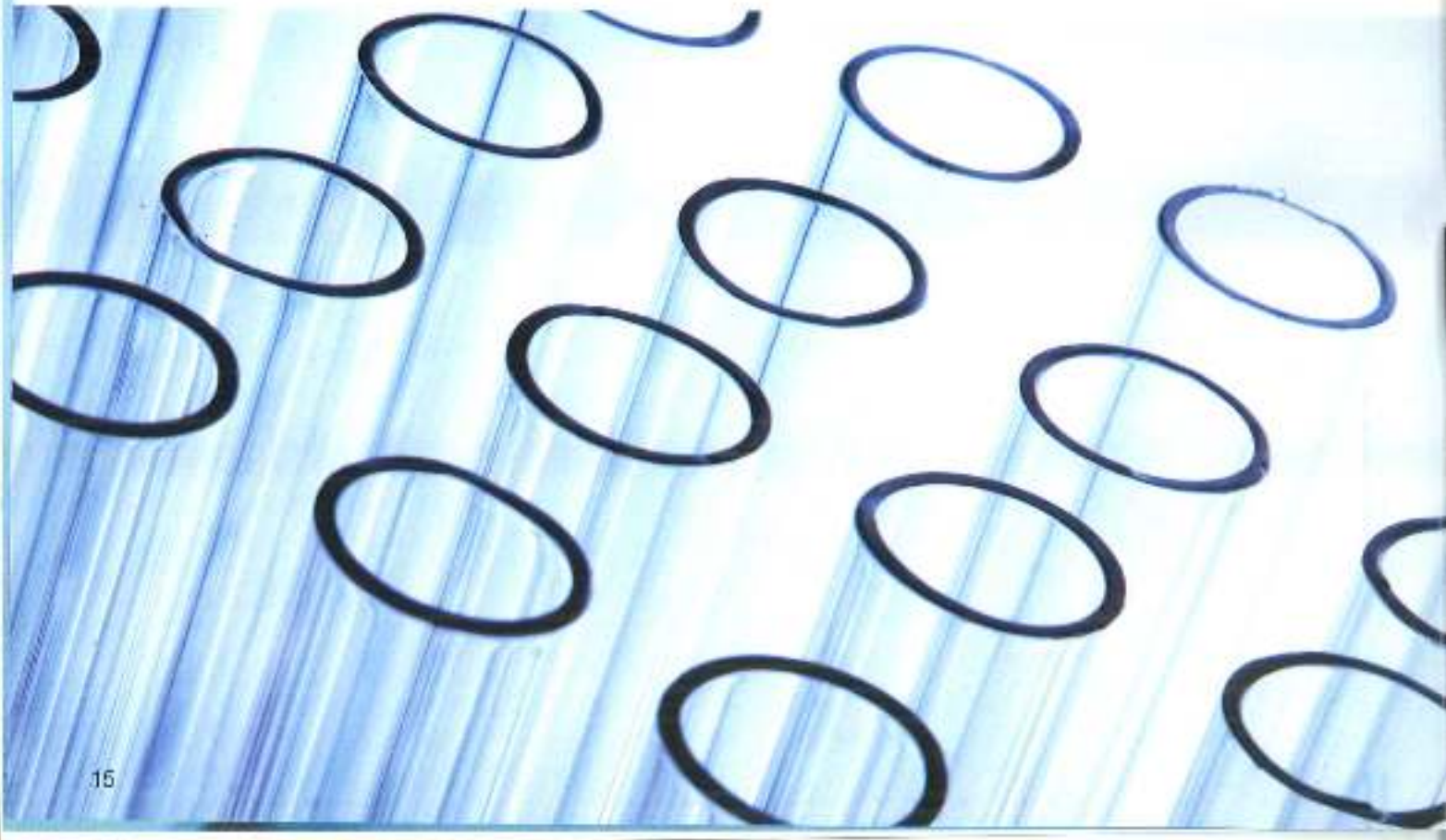
## Enzymes as Life Force

Enzymes are, very simplistically and powerfully put, the "Life Energy" of the body. There are about 3000 enzymes catalyzing some 7000 reactions in the body. They are catalysts for every bodily function and an essential component of all major systems. Vitamins, minerals and hormones must have enzymes to work properly. Life cannot exist without enzymes, they being responsible for the functions of every organ. Every gene's program is associated with the action of enzymes. Enzymes are involved in building, operating and maintaining life. If the body has such a magnificent system to run itself why talk about Enzyme Therapeutics. As you age, there is a reduction in production of enzymes. Tests have shown that 70 year old people have about 1/2 the enzymes of a 20 year old. Once illness or infection has invaded, the older body has to work overtime to produce enzymes needed to overcome the problem. Often it cannot produce enough and chronic disease sets in. This is proved by the fact that many of the modern diseases have been proved to be due to enzyme shortages. Studies indicate that decreased enzymes levels are found in a number of chronic ailments, such as arthritis, diabetes, allergies, skin diseases, cancer, immune deficiencies and many more. This is also the case with genetic deficiencies (3).

| Enzyme Therapeutic | Indication |
|---|---|
| Lipase, Amylase, Protease | Pancreatic insufficiency |
| Porphobilinogen Deaminase | Acute intermittent Porphyria attacks |
| PEG-Uricase | Hyperuricemia in patients with severe gout |
| Recombinant Urate oxidase | Chemotherapy induced hyperuricemia |
| PEG-arginine deiminase | Hepatocellular Carcinoma |
| Phenylalanine Ammonia lyase | Hyperphenylalaninemia |
| Butyrylcholinesterase | Post-surgical apnea |
| Agalsidase Alfa/Agalsidase | Fabry disease |
| Heparin, Trypsin, Chymotrypsin | Multiple Myeloma |
| Chondroitinase | Patients undergoing Vitrectomy |

## Enzyme Therapeutics: Past, Present and Future

Enzyme Therapeutics have been used globally, though in a limited way. An important area of application of Enzyme Therapeutics has been Enzyme Replacement Therapy (3,4). It is the most widely used application and the 10th largest selling class of Biologics (total sales of USD 1.25 billion in 2006). The rationale is supplying an enzyme that is deficient due to a genetic disorder. Examples are shown in Table 1.



Enzyme Therapeutics have been used for some specific diseases, e.g. Elspar (or L-asparaginase) in cancer. Here, the drug converts asparagine to aspartate so that cancer cells are starved of asparagine and die. Proteolytic enzymes such as Papain (brands such as Accuzyme, Ethazyme, Glacase, Kovia) and Trypsin (brands such as Xenaderm and Granuex) have been used in debridement of wounds. A USFDA approved oral drug with brand name "Bromase" containing bromelain is listed in the Physician's Desk Reference in North America and is used as a prostaglandin modulator for inflammation(6).

There are also some Enzyme Therapeutics, listed below, which have been approved recently (2006) by USFDA as drugs.

- Myozyme, an Alglucosidase Alfa, for Pompe disease
- Elaprase, an Idursulfase, for Hunter syndrome

On the horizon are Investigational New Drug (IND) filings with USFDA for Enzyme Therapeutics. Examples are given below:

- A Polyenzyme mix for Cystic Fibrosis patients undergoing Phase III studies under orphan drug and fast track status
- A single enzyme molecule for Hyperphenylalaninemia undergoing Phase II trials
- Drug Zentase for Exocrine pancreatic insufficiency in Phase II trials

All these examples highlight a case for Enzyme Therapeutics as mainstream drugs, and increasingly so. In Europe and Japan, Enzyme Therapeutics have been extensively used for the last 40 years or so. An example is the drug Wobe-Mugos, a combination of systemic enzymes approved in 2000 by USFDA as Orphan Drug for adjunct therapy of multiple Myeloma and has been used successfully in Europe in conjunction with Chemotherapy since 1977. Similarly in South America and Japan, oral enzymes have been used extensively for cardiovascular health and as an anti-inflammatory for over four decades.

In India, well-accepted Enzyme Therapeutics are as follows:

- Pancreatin - For pancreatic insufficiency, GI disorders & immobile life-styles
- Trypsin - Chymotrypsin - Widely used as NSAID for post operative wounds, oedema and Haematoma
- Fungal Diastase + Papain - Indicated for Hyperacidity, Peptic ulcer, Oesophagitis, flatulent Dyspepsia
- Serratopeptidase - Anti-inflammatory, Anti-oedemic, Fibrinolytic For post surgery & infections

## Future of Enzyme Therapeutics as "Drugs of Choice"

A well-documented past, a looking up present can only lead to a bright future. This certainly seems to be the case with Enzyme Therapeutics. Their inherent attributes of being efficacious (specific in action), non-toxic (no LD50) and negligible side effects are well proven. A series of external circumstances are presenting themselves including increased acceptance of biologics, change in mind-set of global pharmaceutical companies towards innovative drugs and a huge body of evidence in favor of Enzyme Therapeutics from Europe, Japan etc. to make Enzyme Therapeutics a front runner for "Drugs of Choice".

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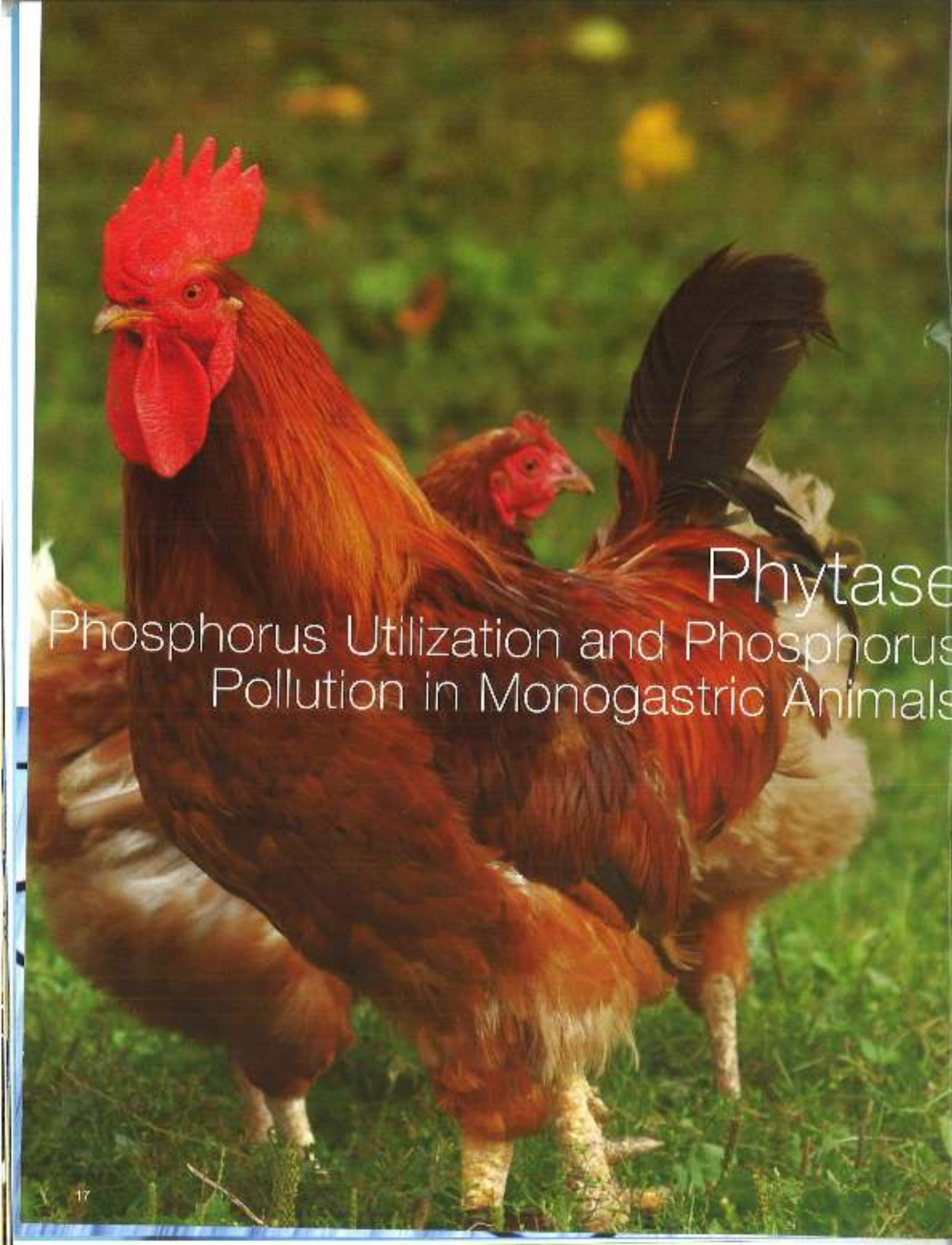
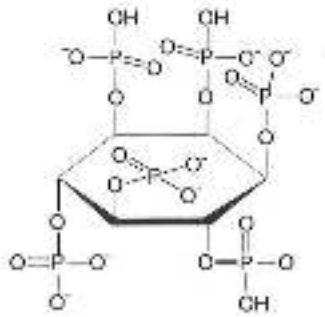
*- Dr. Vardane Sarde*



# Phytase
## Phosphorus Utilization and Phosphorus Pollution in Monogastric Animals

Phytic acid (phytate) is the principal storage form of phosphorus in many plant tissues, especially grains. Phytase is the enzyme that breaks down phytic acid, providing bioavailable phosphorus, calcium and other nutrients. Ruminants, like cattle, readily utilize phytate because of the endogenous phytase produced by rumen microorganisms. Non-ruminants (monogastric animals) like human beings, pigs and poultry do not possess this enzyme. Therefore, phytase can play an important part in the utilization of phosphorus by monogastric animals. Advanced Enzymes, Specialty Enzymes and Biochemicals are major producers and distributors of phytase as well as many other enzymes and enzyme blends for animal feed.

Phosphorus is an essential element for all living organisms. It is an important component of DNA, RNA and many important metabolic processes, such as oxidative phosphorylation. Because of its importance, providing bioavailable phosphorus is



essential to the health, growth and development of all animals, especially monogastric animals like poultry, swine and Homo sapiens. Phosphorus in phytate form is generally not bioavailable to non-ruminant animals since they lack the phytase enzyme. Phytase effectively separates phosphate groups from the phytate molecule, an important function for monogastric animals that primarily feed on grain and other plant foods.[1]

## Animal Feed

Phytase is commonly used as an animal feed supplement for poultry and swine, though other animals may benefit as well. Phytase enhances the nutritional value of plant-based feed, particularly concentrated feed grain. Phytase liberates inorganic phosphate from phytic acid, not only increasing the bioavailability of phosphorus, but improving mineral absorption and protein digestion. As a secondary benefit, phytase helps reduce environmental phosphorus pollution.[1,2]

In most commercial agriculture, non-ruminant livestock such as swine and poultry are mainly fed grains such as soybeans, corn and, more recently, sorghum. Because phytate from these grains is unavailable for absorption, the unabsorbed phytate passes through the gastrointestinal tract, significantly raising the level of phosphorus in manure. Excess phosphorus excretion is an important factor in environmental pollution of soil and groundwater.[3]

## Production Farming

In many areas of the world, livestock production is highly industrialized. This is particularly true in North America and parts of Europe. Production farms owned by large corporations will incorporate millions of pigs, chickens or turkeys in a relatively small area. Combine that with the typical feed for monogastric animals, predominantly concentrated grain, and this results in enormous levels of phosphorus in the manure produced on these corporate farms. As a result, large amounts of phosphorus enter the environment through runoff and direct percolation into ground water. In many cases, sensitive watersheds are affected by these generated pollutants.[3]

## Food Supplementation and Nutrition

Undigested phytic acid is known to decrease mineral availability.[4]

Phytate can bind with multivalent cations to form insoluble complexes, which appear to be resistant to digestion and absorption. Thus bottom line: with high phytate foods, there is a net decrease in the bioavailability of minerals. These include zinc, calcium, iron and potassium in particular.[2,4,5] On the positive side, a study conducted on broiler chicks demonstrated that the addition of phytase to feed reduced the need for supplemental zinc by 40%.[2]

The charged phosphate groups on phytic acid can form complexes with the terminal amino acid of proteins or with free form amino acids. Phytate-mineral-protein complexes also occur. It appears that the ability of phytic acid to form complexes with minerals and protein does reduce the digestion and utilization of these important nutrients in the small intestine of monogastric animals.[1]

Pretreatment of feed with phytase enzyme provides efficient and measurable hydrolysis of phytate. Dietary supplementation of microbial phytase is well established as an effective and practical method of improving phytate digestibility in production animals.[2] Studies indicate a 20-40% improvement in phytate-phosphorus utilization in poultry. Similar work in pigs showed a 40% increase in absorbed phosphorus when phytase was added to a barley soybean meal diet.[3] In a similar study, the inclusion of phytase in a corn-soy diet in swine increased the availability of phytate phosphorus from 15% to 45%.[4]

A study on 10 kg weaning pigs showed an average increase of 17% average daily growth when a combination of phytase, xylanase, amylase and protease was added to feed. Further, there was a substantial increase in the digestibility of calcium and phosphorus in the phytase treated pigs, as well as significant decrease in phosphorus in their manure.[5] Another study on layers demonstrate that phytase improves the utilization of phosphorus and increases egg production in corn-soybean meal diets for laying hens.[6] The results of a second study from the same group indicated not only that phytase improves phosphorus utilization and optimal egg production, but also suggested that older hens may have an even greater need for phytase due to phosphorus deficiency symptoms.[7]

## Pollution

As important as phytase is in the utilization of phosphorus in poultry and swine, the potential to reduce phosphorus pollution is equally important. Many studies demonstrate that supplementation of monogastric animal feed with microbial-derived phytase increases the bioavailability of phytic acid bound phosphate. This facilitates a reduction in the addition of inorganic phosphate to the feed and reduces phosphorus excretion. Harper, et al, found a 21.5% decrease in phosphorus in pig fecal material upon application of phytase to the animals' feed.[8] Other research found decreases ranging from 21% to as high as 41% in pigs, broilers and turkeys.[9][10][11] Clearly, the addition of phytase to the feed of monogastric animals has multiple benefits. First and foremost, increasing the bioavailability of phytate phosphorus is essential to the health, growth and development of monogastric animals. Secondly, phytate forms insoluble complexes with minerals and protein, which in turn make them less available for hydrolysis and eventual absorption. Fortunately, phytase breaks down these complexes increasing the availability and utilization of these essential nutrients, which would otherwise be lost. Finally, the issue of phosphorus pollution is a major concern. The levels of phosphorus in groundwater, sensitive ecosystems or in the fertilizer used to grow our food, are reaching alarming levels. Responsible agricultural practices dictate that all efforts available to reduce phosphorus pollution should be taken. Phytase from Advanced Enzymes and Specialty Enzymes is part of that solution.

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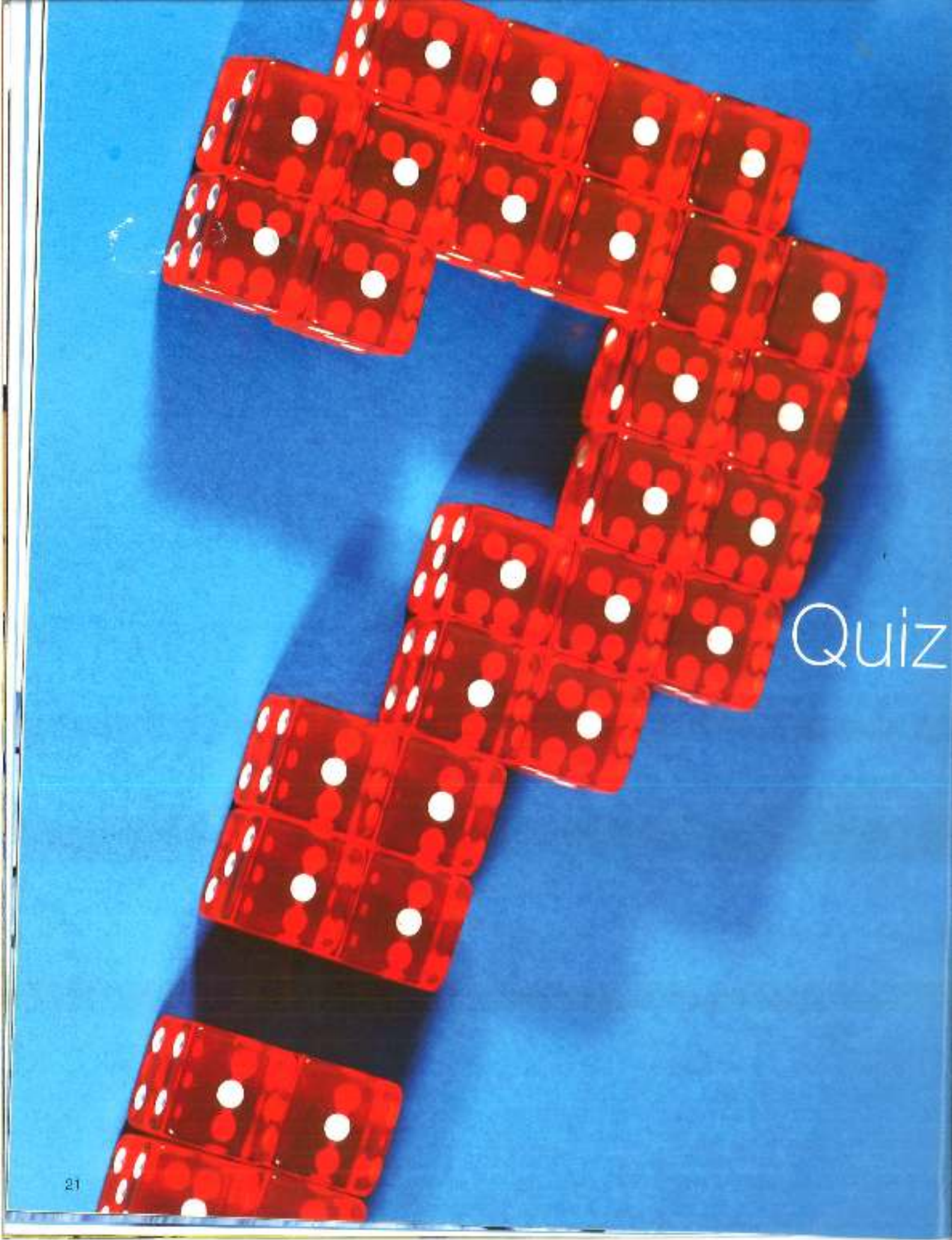

# Quiz

Question 1: The Km Value of enzyme correlates to

(a) Specificity

(b) Activity

(c) Stability

(d) None of Above

Question 2: Lacto Bacillus sporogens produce

(a) Fumaric Acid

(b) Hydrochloric Acid

(c) Lactic Acid

(d) Citric Acid

Question 3: Pepsin digests proteins most rapidly when the PH in stomach is.

(A) 6

(b) 7

(c) 1

(d) 8

Question 4: Bile helps in digestion of fat due to its

(a) Detergent nature

(b) Alkaline nature

(c) Acidic Nature

(d) Hygroscopic nature

Question 5: Which enzyme digests hydrogen peroxide into Oxygen bubbles and water, releasing heat in the process?

(a) Amylase

(b) Catalase

(c) Lipase

(d) Pepsin

Question 6: Amylase is stabilized by ions of

(a) Mercury

(b) Sodium

(c) Potassium

(d) Calcium

Question 7: Saccharomyces Boulardii is a:

(a) Live Bacteria

(b) Live virus

(c) Live yeast

(d) None of Above

Question 8: Enzymes work by lowering

(1) Chemical energy

(2) Free energy

(3) Bond energy

(4) Solvent energy

Question 9: Hemicellulase acts on

(a) Dietary Carbohydrate

(b) Dietary Proteins

(c) Dietary Fibers

(d) None of Above

Question 10: Allosteric inhibition enzymes are brought about by

(a) Change in the energy level

(b) Change in the conformation

(c) Change in PH

(d) Change in Temp.

Send in your answers to info@enzymeindia.com

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