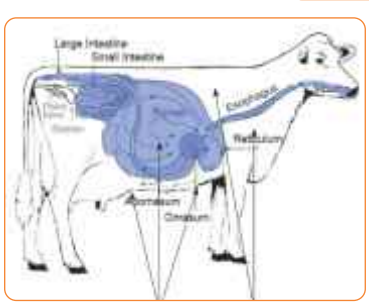
Contents



CONTRIBUTORS

Rohit Chauhan
Business Development

Satish Painuly
QA & QC Department

Dharmender Pathak
R&D Department

Dr. Archana Prakash
R&D Department

Monika Chaudhary
HR & Admin Department

MESSAGE FROM THE MANAGING DIRECTOR

I would like to take this opportunity to wish everyone a happy and healthy festive season. First half of the financial year 2016-17 is over and October marks the beginning of the second half.

Catalysts Group has become a high-achieving, result-oriented organizational culture that puts the customer at the centre of everything it does. This customer centric approach has enabled Catalysts to continuously evolve its product, team & structure. The driver remains a passion to be the Numero Uno in whatever it does.

To survive and grow we have to be the best at what we do, becoming thought leaders and discovering opportunities in every challenge. Through our in-depth knowledge of enzymes and market trends and our expertise in R&D, we have had the courage and tenacity to develop products that create both immediate and lasting value for our clients.
We are motivated by delivering effective solutions that make a difference. We are passionate about research, not for its own sake, but for the ways in which it can solve complex production problems of our customers. Our research and customer support teams have an exceptional depth of expertise in technology and industry operations.

Client satisfaction is our ultimate aim and our highest priority. We provide unique insights and add significant, quantifiable value to their businesses. Over the years we have played a pivotal role in driving success, supporting growth and facilitating change for our clients.

Change is the only thing constant! I strongly believe in this and would like all of you to also imbibe this value. Each individual at Catalysts is encouraged to evolve and grow. Keep learning and keep growing should be our motto!

Munish Madaan

MESSAGE FROM THE DIRECTOR

Dear Friends,

Here at Catalysts every year one one hand we celebrate the festivals together and on the other hand our sales & production team is all geared up to take challenges of new sugar & molasses season. We have increased our technical sales team recently in Brewing, Grain & Molasses verticals with primary focus on offering more enhanced technical services to respective industries. Due to raw material prices steeping high the industry bleeds.

We are happy that with time industry has become more open to innovations in their processes. This motivates our R&D to work on challenges to offer solutions across regions & countries. Some of our recent innovations like enzymes for molasses preservation have been very well accepted by the industry. Through our international tie-ups we have been able to launch yeast for high gravity fermentation in molasses where too the product has been accepted very well both technically & commercially by the industry.

We at Catalysts are committed to innovate & offer you the best solution to your industry related problems and this has only been possible because of your guidance & support.

Wishing you & your family a very Happy Dussehra & Happy Diwali.

Aditya Malhotra

FUNCTION OF ENZYMES IN BREWING

Rohit Chauhan, Business Development Department

THE NATURE OF ENZYMES

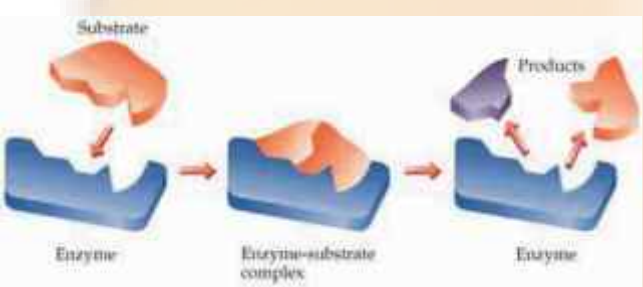
Enzymes are proteins with a special structure capable of accelerating the breakdown of different substrates. They act as catalysts to increase the speed of a chemical reaction without themselves undergoing any permanent chemical change. They are not used up in the reaction or appear as reaction products. The basic enzymatic reaction can be represented as follows:

Substrate + Enzyme ↔ Product(s) + Enzyme.

Energy is required for chemical reactions to proceed. The energy is called the energy of activation. It is the magnitude of the activation energy that determines just how fast the reaction will proceed.

HOW ENZYMES WORK

Enzymes bind temporarily to substrate of the product they catalyse. In doing so, they lower the amount of activation energy needed enabling the reaction to proceed at more quickly at lower temperatures.



In order to do its work, an enzyme must combine-even if ever so briefly-with at least one of the reactants. In most cases, the forces that hold the enzyme and its substrate are non-covalent, being an assortment of:

- Hydrogen bonds
- Ionic bonds
- Hydrophobic interactions

Most of these interactions are weak and successful binding of enzyme and substrate requires that the two molecules are able to approach each other closely over a broad surface. The substrate molecule binds to the enzyme like a key in a lock. This means that the structure of the substrate has to match the shape of the enzyme and explains the remarkable specificity of most enzymes. The necessity for a close fit between enzyme and substrate explains how the enzyme can be inhibited by molecules with a similar structure.

Many enzymes require the presence of an additional, non-protein, co-factor.

- Some of these are metal ions such as Zn_2+ (the co-factor for alcohol dehydrogenase), Cu_2+, Mn_2+, K+, and Na+
- Some co-factors are small organic molecules called co-enzymes-for example the B vitamins

TEMPERATURE SENSITIVITY

Like most chemical reactions, the rate of an enzyme-catalyzed reaction increases as the temperature is raised. A 10°C rise in temperature will increase the activity of most enzymes by 50 to 100%. Variations in reaction temperature as small as 1-2° may introduce changes of 10 to 20% in the results.

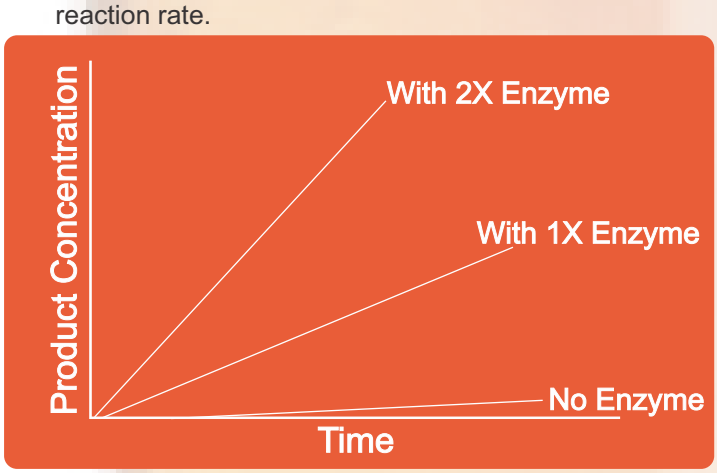
In the case of enzymatic reactions, this is complicated by the fact that high temperatures adversely affect many enzymes. The reaction rate increases with temperature to a maximum level,

then abruptly falls off with further increase of temperature. Many enzymes start to become denatured at temperatures above 40°C.

Over a period of time, enzymes will be deactivated at even moderate temperatures. Storage of enzymes at 5°C or below is generally the most suitable.

ENZYME CONCENTRATION

With an excess concentration of substrate, such as starch in a brewers wort, there is a linear effect of increasing the enzyme concentration upon the reaction rate.



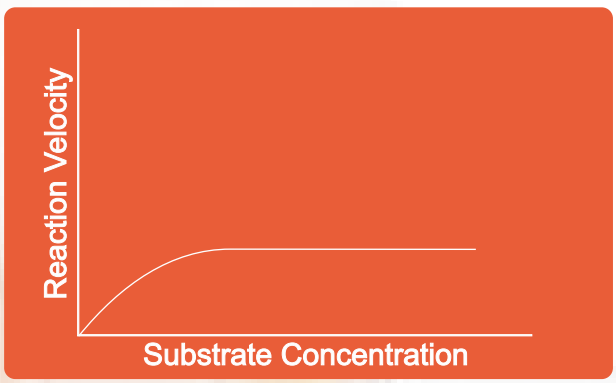
Hence the if all other factors are kept constant, malts with higher enzymic power will break down the starch faster.

The amount of enzyme present in a reaction is measured by the activity it catalyses.

SUBSTRATE CONCENTRATION

It has been shown that if the amount of the enzyme is kept constant and the substrate concentration is then gradually increased, the reaction velocity will increase until it reaches a maximum. After this point, increases in substrate concentration will not increase the velocity.

It is thought that when this maximum velocity had been reached, all of the available enzyme has been converted to the enzyme substrate complex. This point on the graph is designated Vmax. This information can be used to calculate enzyme activity in terms of rate of reaction.



In addition to temperature and pH there are other factors, such as ionic strength, which can affect the enzymatic reaction. Each of these physical and chemical parameters must be considered and optimised in order for an enzymatic reaction to be accurate and reproducible.

BIOCHEMICAL CHANGES DURING BREWING

Enzymes are essential in catalyzing the biochemical changes, which occur in the brewing process.

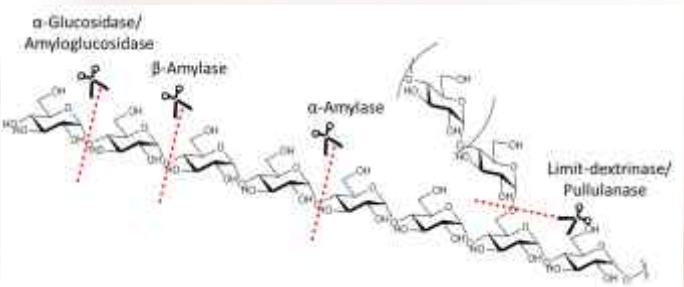
There are two principal processes of interest to the brewer:

- The break down the carbohydrate, principally starch in malted barley to sugars
- The fermentation of sugars and other nutrients under anaerobic conditions by yeast to release energy and producing ethanol as a metabolic by-product. These biological reactions are catalyzed by enzymes from the barley and yeast respectively. Every organism is obliged to produce all the enzymes needed to break down its component molecules.

STARCH HYDROLYSIS

The principal enzyme reaction involved in mashing is the hydrolysis of starch to sugars by alpha- and beta-amylase. Before enzyme hydrolysis can occur it is necessary to exceed the starch gelatinisation

temperature of malt. Therefore it is necessary to select the optimum conditions for the saccharifying enzymes to operate. This is achieved by stabilizing the enzymes in a number of ways:



- Optimising pH at mashing (usually between pH5 and 6)
- Adding calcium ions to stabilize the enzyme
- Using thick mash (high concentration substrate to insulate the enzymes against denaturing)
- Optimising temperature to favor the activity of both the alpha and beta amylase.

The amylase enzymes are able to break the alpha-1,4 links in amylose and amylopectin to give a mixture of glucose, maltose, maltotriose and higher sugars called dextrins, which are unfermentable, to give a wort (malt derived sugar solution) which is about 70% fermentable.

- alpha-amylase produces random hydrolyses of starch to dextrins
- beta-amylase attacks the starch and dextrins from the reducing end, stripping off pairs of sugars molecules (maltose)

By varying the mashing temperature it is possible to preferentially favour one enzyme reaction over the other and hence influence the fermentability of the wort, with the lower temperatures giving higher fermentable worts.

EFFECT OF MASH TEMPERATURE ON WORT FERMENTABILITY (ALL MALT MASH)

Mash Temp °c	% Extract	% Fermentability	Attenuation Limit For 1040 Wort (10°P)
60	75.6	76.2	2.7
65.5	74.2	69.7	3.2
68.3	74.0	65.3	3.7

BETA-GLUCAN BREAKDOWN

As well as starch there are a number of non-starch barley polysaccharides. The most significant non-starch polysaccharide in barley and malt is beta-glucan which makes up more than 75% of the cell wall. The molecule has a distinctive linear structure in with roughly 70% beta-1,4 linkages and 30% beta-1,3 linkages.

Most beta-glucan is water soluble, but a proportion is bound co-valently to cell wall proteins. If there is insufficient degradation of the cell walls, then enzymic access to the protein and starch will be restricted, and the extract from the malt reduced. Although much of the necessary beta-glucanase activity occurs during malting, there is inevitably some survival of cell wall material (even in the most fully modified malt).

This will be exacerbated if adjuncts such as barley and wheat are also used. Consequently it is necessary to ensure the continued activity of beta-glucanase during mashing, since the release of beta-glucan will continue through the activity of beta-glucan solubilase which is more heat stable than the malt beta-glucanase which breaks down the beta-glucan structure.

TEMPERATURE OF BETA GLUCAN ENZYME ACTIVITY			
ENZYME	**pH OPTIMUM**	**OPTIMUM TEMP.**	**TEMP. INACTIVE**
Endo beta 1-4 Glucanase	4.5-4.8	37-45°C	55°C
Endo barley beta Glucanase	4.7-5.0	40°C	63°C
Beta glucan Solubilase	6.3	60°C	73°C

The results of the different optimum temperature can have an effect on the viscosity and hence the filterability of wort and beer.

EFFECT OF MASHING TEMPERATURES ON THE RELEASE OF BETA GLUCAN			
Temp °C	**Wort beta glucan (mg/l)**	**Wort viscosity cp**	**Wort filtration rate volume in 30 mins**
45	1.2	1.20	277
55	2.8	1.35	178
65	6.3	1.63	133
75	9.7	2.01	78

If the large viscous beta-glucan molecules are not broken down during malting or mashing other process problems can also occur:

- Reduced extract recovery
- High wort viscosit
- Poor run off performance
- Beer filtration problems
- Beer haze problems

The high molecular weight beta-glucans released by beta-glucan solublilase contribute to wort viscosity and poorer extract recovery. Most brewers are very careful in selecting malt with low beta-glucan levels and beta-glucan degradation occurs during malting.

However most initial mash temperatures are at or above the maximum stability temperature of the malt beta glucanase enzymes and it is common practice in many breweries to add exogenous beta-glucanase to decrease wort and beer viscosity and to improve filterability.

The effects of enzyme addition on the breakdown of beta-glucan can be shown by an increasing in filter flow rate and decrease in wort viscosity. The effect will be more noticeable with higher concentrations of unbroken beta-glucans when using for example raw (un-malted) barley adjunct.

HYDROLYSIS OF PROTEINS AND POLYPEPTIDES

While about 95% of the starch from malt is solubilised by the end of mashing, only about 35-40% of the malt protein (TN-total nitrogen) is solubilised. This is referred to as the TSN (total soluble nitrogen) in an un-boiled wort.

The permanently soluble nitrogen (PSN) is the nitrogenous material which remains in the wort after wort boiling (i.e. is not precipitated as break). *The PSN is usually calculated as*

TSN x 0.94

The principal groups of enzymes involved in the breakdown of malt proteins are Endoproteases which break the large protein molecules into relatively large polpeptide chains, and the Exopeptidases which attack the polypeptides from a specific end stripping off small units to produce amino acids.

ENDOPEPTIDASES

They have a relatively low optimum temperature and hence with high temperature mashing (e.g. 65°C isothermal mashing) most of the protein breakdown will have taken place during the malting process and randomly attack the protein chain.

Optimum conditions	
pH	3.9-5.5
Temperature	45-50°C
Inactivation Temp.	70°C

EXOPEPTIDASES

They are able to withstand higher temperatures and release the amino acids from the prolypeptide chains.

There are two principal groups of Exopeptidase enzymes:

- **Carboxypeptidase** which attacks the proteins from the carbonyl end. This enzyme is not present in raw barley, but is rapidly produced during steeping and is active at normal mash pH.

Optimum conditions	
pH	3.9-5.5
Temperature	45-50°C
Inactivation Temp.	70°C

- **Aminopeptidase** which attacks the proteins from the amino end, is much less active at mash pH and does not play a significant role in protein breakdown during mashing.

Optimum conditions	
pH	4.8-5.2
Temperature	50°C
Inactivation Temp.	>70°C

Most of the proteolysis occurs during malting. It is impossible to completely compensate for a nitrogen deficiency in malt by introducing a prolonged mash stand at < 50°C without adding exogenous enzymes.

TYPICAL TYPES OF PROTEIN MATERIAL FOUND IN WORT

1. Proteins
2. Polypeptides
3. Peptides
4. Amino Acids

FERMENTATION

Most living organisms respire aerobically, converting sugars to carbon dioxide and water releasing the energy bound by photosynthesis in the carbohydrate (sugars) molecules.

However some micro-organisms, including yeast, are able to respire anaerobically, but under anaerobic conditions they can only partially break down the sugar molecules to ethanol to release energy in the form of ATP (Adenosine Triphosphate).

The role of yeast in the fermentation is that of a living catalyst, effecting the reaction without becoming part of the finished product. During the course of the fermentation the yeast cells grow and replicate up to 5 times.

Although the yeast gains its energy from the sugar, which it converts to alcohol it can only utilise simple sugars. The sugars are taken up in a specific order, with the monosaccharides, glucose and fructose used first, together with sucrose. Although the latter is a disaccharide, it behaves like a monosaccharide since it is broken down to glucose and fructose outside the cell through the action of the yeast enzyme invertase.

Once the wort glucose level falls, the yeast starts to use the disaccharide, maltose, which is usually the most abundant sugar in brewers wort. Maltose has to be transported into the cell, where it is broken down to glucose. Lastly most yeast strains can utilise the trisaccharide, maltotriose, but only slowly.

Brewing strains of yeast cannot generally ferment the longer chained or branched sugars (called dextrins) which persist in to the finished beer as unfermentable extract to give the beer body and mouthfeel.

As well as sugars, yeast requires nitrogen, which in wort comes from the malt in the form of soluble amino nitrogen. A healthy fermentation yeast requires more than 160 mg/l of soluble nitrogen.

If there is insufficient soluble nitrogen, for example when high cereal or sugar adjunct are used, then additional nitrogen may be required in the form of simple ammonium salts.

Source: IBD Journals

Production & Use of Microbial Enzymes for Dairy Processing

Satish Painuly, QA & QC Department

This article presents a review of the microbial enzymes used in dairy applications; primarily, milk-clotting enzymes or rennets, recombinant fungal and bacterial rennets for cheese manufacture, and fungal lactases for the manufacture of some milk products with reduced content of lactose. Other dairy enzymes include proteinases for accelerated cheese ripening for good flavor and textural development, proteases to reduce allergic properties of cow milk products for infants, and lipases for the development of lipolytic flavours in speciality cheeses.

Indians are known to be lovers of milk and its products. As a sequel to white revolution, India has surged ahead to become the largest milk producer in the world, the production figure touched to 74 million tons for the year 1998. With growing urbanization, demand for processed dairy foods has increased considerably, in particular demand for different cheese varieties and low-lactose milk due to increasing intolerance of human beings to lactose in milk and other milk products. For improving the quality of milk and milk products, a number of different enzymes from microbial as well as from non-microbial sources have potential applications in dairy processing.

The use of rennet in cheese manufacture was among the earliest applications of exogenous enzymes in food processing, dating back to approximately 6000 B.C. In 1994, the total production of cheese was 8000 metric tons against a total demand of 9000 metric tons. The projected demand by 2000 A.D. is around 30,000 metric tons. The use of rennet, as an exogenous enzyme, in cheese manufacture is perhaps the largest single application of enzymes in food processing. In recent years, proteinases have found additional applications in dairy technology, for example in acceleration of cheese ripening, modification of functional properties and preparation of dietic products. Principal among some enzymes that have important and growing applications are lipases and b-galactosidases. Enzymes with limited applications include glucose oxidase, superoxide dismutase, sulphydryl oxidase, etc. The increasing use of enzymes to produce specific products with characteristic attributes can be emphasized by the world-wide sale of industrial enzymes approximating to US $ 1.6 billion, which is expected to reach US $ 3.0 billion by the year 2008. Almost 45% of this is shared by the food industry and the remaining is shared by detergent (34.4%), textile (11%), leather (2.8%), pulp and paper (1.2%) industries and other industries (5.6%) excluding enzymes for use in diagnostics and therapeutics.

India being the highest producer of milk in the world, and consequently the surplus availability of milk in our country has triggered the food and dairy industry to convert the liquid milk into value-added products using biochemical and enzymatic processes.

MICROBIAL RENNETS IN DAIRY APPLICATIONS

Animal rennet (bovine chymosin) is conventionally used as a milk-clotting agent in dairy industry for the manufacture of quality cheeses with good flavor and texture. Owing to an increase in demand for cheese production world wide – i.e. 4% per annum over the past 20 years, approximating 13.533 million tons - coupled with reduced supply of calf rennet, has therefore led to a search for rennet substitutes, such as microbial rennets. At present, microbial rennet is used for one-third of all the cheese produced world wide.

Rennin acts on the milk protein in two stages, by enzymatic and by nonenzymatic action, resulting in coagulation of milk. In the enzymatic phase, the resultant milk becomes a gel due to the influence of calcium ions and the temperature used in the process4. Many microorganisms are known to produce rennet-like proteinases which can substitute the calf rennet. Microorganisms like Rhizomucor pusillus, R. miehei, Endothia parasitica, Aspergillus oryzae, and Irpex lactis are used extensively for rennet production in cheese manufacture. Extensive research that has been carried out so far on rennet substitutes has been reviewed by several authors. Different strains of species of Mucor are often used for the production of microbial rennets. Whereas best yields of the milk-clotting protease from Rhizomucor pusillus are obtained from semisolid cultures containing 50% wheat bran, R. miehei and Endothia parasitica are well suited for submerged cultivation. Using the former, good yields of milk-clotting protease may be obtained in a medium containing 4% potato starch, 3% soybean meal, and 10% barley. During growth, lipase is secreted together with the protease. Therefore, the lipase activity has to be destroyed by reducing the pH, before the preparation can be used as cheese rennet.

LACTASE IN DAIRY INDUSTRY

Lactose, the sugar found in milk and whey, and its corresponding hydrolyze, lactase or b-galactosidase,

have been extensively researched during the past decade. This is because of the enzyme immobilization technique which has given new and interesting possibilities for the utilization of this sugar. Because of intestinal enzyme insufficiency, some individuals, and even a population, show lactose intolerance and difficulty in consuming milk and dairy products. Hence, low-lactose or lactose-free food aid program is essential for lactose-intolerant people to prevent severe tissue dehydration, diarrhoea, and, at times, even death. Another advantage of lactase-treated milk is the increased sweetness of the resultant milk, thereby avoiding the requirement for addition of sugars in the manufacture of flavored milk drinks. Manufacturers of ice cream, yoghurt and frozen desserts use lactase to improve scoop and creaminess, sweetness, and digestibility, and to reduce sandiness due to crystallization of lactose in concentrated preparations. Cheese manufactured from hydrolysed milk ripens more quickly than the cheese manufactured from normal milk.

Technologically, lactose crystallizes easily which sets limits to certain processes in the dairy industry, and the use of lactase to overcome this problem has not reached its fullest potential because of the associated high costs. Moreover, the main problem associated with discharging large quantities of cheese whey is that it pollutes the environment. But, the discharged whey could be exploited as an alternate cheap source of lactose for the production of lactic acid by fermentation. The whey permeate, which is a by-product in the manufacture of whey protein concentrates, by ultrafiltration could be fermented efficiently by Lactobacillus bulgaricus. Lactose can be obtained from various sources like plants, animal organs, bacteria, yeasts (intracellular enzyme), or molds. Some of these sources are used for commercial enzyme preparations. Lactase preparations from A. niger, A. oryzae and Kluyveromyces lactis are considered safe because these sources already have a history of safe use and have been subjected to numerous safety tests. The most investigated E. coli lactase is not used in food processing because of its cost and toxicity problems.

PROPERTIES OF LACTASE

The properties of the enzyme depend on its source. Temperature and pH optima differ from source to source and with the type of particular commercial preparation. Immobilization of the enzymes, method of immobilization, and type of carrier can also influence these optima values. In general, fungal lactase have pH optima in the acidic range 2.5-4.5, and yeast and bacterial lactases in the neutral region 6-7 and 6.5-7.5, respectively. The variation in pH optima of lactases makes them suitable for specific applications, for example fungal lactases are used for acid whey hydrolysis, while yeast and bacterial lactases are suitable for milk (pH 6.6) and sweet whey (pH 6.1) hydrolysis. Product inhibition, e.g. inhibition by galactose, is another property which also depends on the source of lactase. The enzyme from A. niger is more strongly inhibited by galactose than that from A. oryzae. This inhibition can be overcome by hydrolysing lactose at low concentrations by using immobilized enzyme systems or by recovering the enzyme using ultrafiltration after batch hydrolysis. Lactase from Bacillus species are superior with respect to thermostability, pH operation range, product inhibition, and sensitivity against high-substrate concentration. Thermostable enzymes, able to retain their activity at 60°C or above for prolonged periods, have two distinct advantages viz. they give higher conversion rate or shorter residence time for a given

conversion rate, and the process is less prone to microbial contamination due to higher operating temperature. Bacillus species have a pH optima of 6.8 and a temperature optima of 65°C. Its high activity for skim milk, and less inhibition by galactose has made it suitable for use as a production organism for lactase.

The enzymatic hydrolysis of lactose can be achieved either by free enzymes, usually in batch fermentation process, or by immobilized enzymes or even by immobilized whole cells producing intracellular enzyme. Although numerous hydrolysis systems have been investigated, only few of them have been scaled up with success and even fewer have been applied at an industrial or semi-industrial level. Several acid hydrolysis systems have been developed to industry-scale level. Large-scale systems which use free enzyme process have been developed for processing of UHT-milk and processing of whey, using K. lactis lactase (Maxilact, Lactozyme). Several commercial immobilized systems have been developed for commercial exploitation. Snamprogretti process of industrial-scale milk processing technology in Italy is one such working systems. They make use of fibre-entrapped yeast lactase in a batch process, and the milk used is previously sterilized by UHT. For pilot plants, there are three other processes designed and developed to handle milk; (i) by Gist-Brocades, Rohm GmbH (Germany), and (ii) by Sumitomo, Japan. These are continuous processes with short residence times. Processing of whey UF-permeate is accomplished by the system developed by Corning Glass, Connecticut, Lehigh, Valio and Amerace corp. The process by Corning Glass is being applied at commercial scale in the bakers yeast production using hydrolysed whey.

PROTEINASES AND PEPTIDASES

Proteolysis is characteristic of most cheese varieties and is indispensable for good flavour and textural development. Proteinases used in cheese processing include (i) plasmin, (ii) rennet, and (iii) proteinases (cell wall and/or intracellular) of the starter and nonstarter bacteria. Approximately 6% of the rennet added to cheese milk remains in the curd after manufacture and contributes significantly to proteolysis during ripening. Combinations of individual neutral proteinases and microbial peptidases intensified cheese flavor, and when used in combinations with microbial rennets reduced the intensity of bitterness caused by the latter. Acid proteases in isolation cause intense bitterness.

Various animal or microbial lipases gave pronounced cheese flavour, low bitterness and strong rancidity, while lipases in combination with proteinases and/or peptidases give good cheese flavour with low levels of bitterness. In a more balanced approach to the acceleration of cheese ripening using mixtures of proteinases and peptidases, attenuated starter cells or cell-free extracts (CFE) are being favored.

PROTEOLYTIC ENZYMES OF LACTIC ACID BACTERIA IN FERMENTED MILK PRODUCTS

The proteolytic system of lactic acid bacteria is essential for their growth in milk, and contributes significantly to flavor development in fermented milk products. The proteolytic system is composed of proteinases which initially cleaves the milk protein to peptides; peptidases which cleave the peptides to small peptides and amino acids; and transport system responsible for cellular uptake of small peptides and amino acids. Lactic acid bacteria have a complex proteolytic system capable of converting milk casein to the free amino acids and peptides necessary for their growth. These proteinases

include extracellular proteinases, endopeptidases, aminopeptidases, tripeptidases, and proline-specific peptidases, which are all serine proteases. Apart from lactic streptococcal proteinases, several other proteinases from nonlactostreptococcal origin have been reported26. There are also serine type of proteinases, e.g. proteinases from Lactobacillus acidophillus, L. plantarum, L. delbrueckii sp. bulgaricus, L. lactis, and L. helveticus. Aminopeptidases are important for the development of flavor in fermented milk products, since they are capable of releasing single amino acid residues from oligopeptides formed by extracellular proteinase activity.

OTHER DAIRY ENZYMES

Other enzymes used for dairy food application include: (i) proteases to reduce allergic properties of cow milk products for infants, and (ii) lipases for development of lipolytic flavors in speciality cheeses. The functional properties of milk proteins may be improved by limited proteolysis through the enzymatic modification of milk proteins. An acid-soluble casein, free of off flavor and suitable for incorporation into beverages and other acid foods, has been prepared by limited proteolysis. The antigenicity of casein is destroyed by proteolysis, and the hydrolysate is suitable for use in milk-protein-based foods for infants allergic to cow milk.

Lipolysis makes an important contribution to swiss cheese flavors, due mainly to the lipolytic enzymes of the starter cultures. The characteristic peppery flavor of Blue cheese is due to short-chain fatty acids and methyl ketones. Most of the lipolysis in Blue cheese is catalysed by Penicillium roqueforti lipase, with a lesser contribution from indigenous milk lipase.

The NOVO process for production of enzyme modified cheese (EMC) uses medium-aged cheese which is emulsified, homogenized, and pasteurized, after which ‘palatase’ (a lipase from R. miehei) is added, with or without a proteinase, and the blend is ripened at a high temperature for one to four days. The mixture is reheated, a paste results which is suitable for inclusion in soups, dips, dressings, or snack foods. EMC technology has been developed to produce a range of characteristic cheese flavors and flavor intensities, for example swiss, blue, cheddar, provolo-nemor or romano, suitable for inclusion at low levels in many products. The claims that exogenous enzymes are effective in accelerating ripening have not led to their wide-spread use, possibly due to their high cost, difficulties in distributing them uniformly in the curd, and the possible danger of over-ripening the cheese.

Reference: S. Neelakantan, A. K. Mohanty and Jai K. Kaushik

Turning Waste into Food: Cellulose Digestion

Dharmender Pathak, Research & Development Department

INTRODUCTION

Fiber constitutes an essential element in the human diet. It has been shown to prevent cholesterol absorption, heart disease and help control diabetes. The National Academy of Sciences Institute of Medicine recommends the adult male consume at least 38 grams of soluble fiber per day – the only kind of fiber humans can digest. The other more abundant type of fiber, insoluble fiber, passes through the human digestive system virtually intact and provides no nutritional value.

What if humans could digest fiber? Cellulose, the main type of insoluble fiber in the human diet, also represents the most abundant organic compound on Earth. Almost every plant has cell walls made from cellulose, which consists of thousands of structurally alternating glucose units (Fig. 1). Cellulose contains just as much energy as starch and it is only possible to use that energy by burning wood and other cellulose materials. However, if that energy were physiologically available, humans could lower their food consumption and produce much less digestive waste than they currently do.

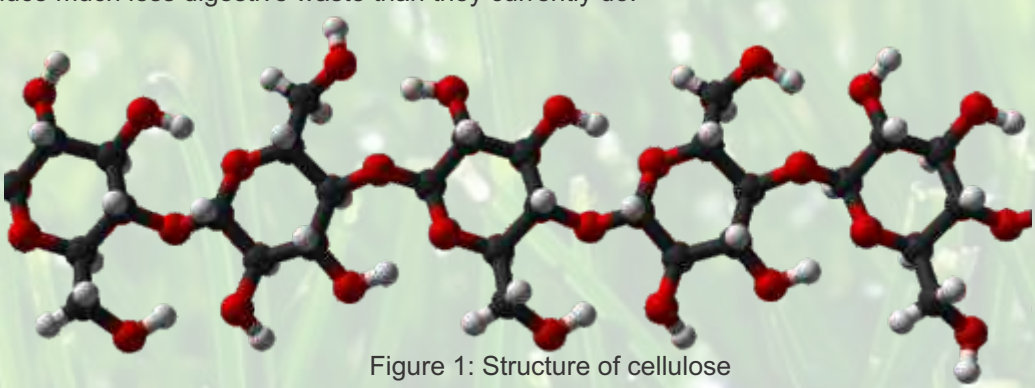


Figure 1: Structure of cellulose

THE HUMAN DIGESTIVE SYSTEM

Disregarding cellulose digestion, human digestion is still a very efficient process. As soon as food enters the mouth, saliva glands start secreting digestive enzymes and lubricants. Amylase breaks down starches in the mouth into simple sugars and teeth grind up the food into smaller chunks for further digestion. After swallowing the food, hydrochloric acid and various enzymes work on the food in the stomach for 2 to 4 hours, during which the stomach absorbs glucose, other simple sugars, amino acids, and some fat-soluble substances.

Although the human digestive system is quite efficient, also suffer from various other enzyme or hormone deficiencies that affect digestion and absorption, such as diabetes. The human mouth, stomach, and liver can secrete enzymes to digest almost every type of carbohydrate except cellulose, which is essential to a herbivore's survival. In the case of lactose intolerance, lactase supplements can easily rectify the deficiency, so what rectifies the inability to digest cellulose?

RUMINANTS AND TERMITES

The intestine of ruminant animals such as goats, sheep, bison, buffalo and antelope – are very similar to human intestines in their form and function (Fig 2). The key to specialized ruminant digestion lies in the rumen. Ruminants also secrete saliva as the primary step in digestion, but unlike humans, they swallow the food first only to regurgitate it later as 'cud' for chewing. Ruminants have multi-chambered stomachs, and food particles are made small enough to pass through the reticulum chamber into the rumen chamber, where special bacteria secrete the necessary enzymes to break down the various forms of cellulose for digestion and absorption.

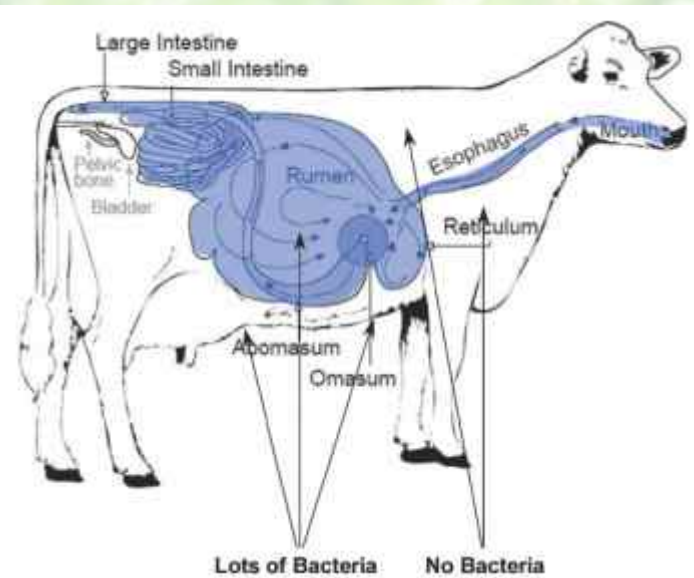


Figure 2: The ruminant digestive system

Some forms of cellulose are more complex and harder to break down than others and some of the microbes in the rumen, such as Fibrobacter succinogenes, produce cellulase that breaks down the more complex forms of cellulose in straw while others such as Ruminococci produce extracellular cellulase that hydrolyzes the simpler amorphous type of cellulose. Conveniently, cellulose hydrolysis produces several byproducts, such as cellobiose and pentose disaccharides, which are useful to rumen microbes. The reactions produce other byproducts such as methane, which is eventually passed out of the ruminant. Thus, the microbes and ruminants live symbiotically so that the microbes produce cellulase to break down cellulose for the ruminants while gaining a food source for their own sustenance.

The various microbes within ruminants may hydrolyze certain types of cellulose, but ruminants still cannot eat wood or cotton. Termites, on the other hand, can feed on various types of wood which for a long time was owed to the microorganisms that lived inside their bodies to digest cellulose, but research in the late 1990s showed that certain types of termites had the ability to produce enough cellulases and xylanases in the midgut. However, other species of termites do not have the capacity to produce enough cellulases, and must depend on microbes from the domains Archaea, Eubacteria and Eucarya to break down cellulose. Both protists and fungi are attributed to the production of supplementary enzymes, but their specific roles and mechanisms are still being debated because isolating pure cultures has proven technically difficult. Despite the ubiquity of these microbes and the benefits they bring to ruminants and termites, research has yet to fully elucidate their mechanisms.

CURRENT TECHNOLOGIES TO CONVERT CELLULOSE INTO ENERGY

People have long been interested in tapping into the energy in cellulose. However, most companies and research groups are only focused on ways to harness that energy as biofuel and not as food. Major research is aimed at converting cellulosic material into ethanol, although that process is still inefficient and requires refinement. In this process, cellulose must first be hydrolyzed into smaller sugar components such as glucose, pentose or hexose before it can be fermented into bioethanol. One method uses acids to hydrolyze cellulose

but this can destroy much of the sugar in the process. Another way to hydrolyze cellulose is using the enzymes produced by microbes to break down cellulose. However, enzymes have biological limitations and implement natural feedback inhibition that poses a problem for industrial manufacturing. Other technical barriers to efficient enzymatic hydrolysis include the low specific activity and high cost of current commercial enzymes and a lack of understanding of the mechanisms and biochemistry of the enzymes.
Companies and governments all over the world are eager to invest heavily in research to turn biomass into biofuel, which could bring enormous benefits to the world economy and environment. Biomass is readily available, biodegradable, and sustainable, making it an ideal choice as a source of energy for both developed and developing countries. This could also help reduce waste problems plaguing society today. The United States produces 180 million tons of municipal waste per year, and about fifty percent of this is cellulosic and could potentially be converted into energy with the right technology.

CELLULOSE DIGESTION IN HUMANS

The benefits of turning cellulose into biofuel are just as relevant when considering engineering humans to digest cellulose as a food source. Perhaps in the future humans could serve as the machine for extracting energy from cellulose, especially since the enzymes used to hydrolyze cellulose are hard to isolate in large quantities for industrial use. Termites and ruminants serve as a great example of how organisms can use microbes effectively. Termites themselves are tiny creatures, but as a colony, they can break down houses and entire structures. A healthy human digestive system already carries an estimated 1 kg of bacteria, so adding a couple of extra harmless types should not pose a problem.

A simpler solution would be to take supplements similar to the ones used to treat lactose intolerance. Cellulose can be broken down and absorbed as glucose, by supplementing the right enzymes to work in the human stomach. Perhaps the easiest solution for cellulose indigestion is to extract the appropriate enzyme from the right microbes and use them as food supplements. Additionally, since the process would occur inside the human body, the limitations that posed a problem for commercial hydrolysis of cellulose would become necessary biological controls.

CONCLUSION

Vegetables, which is severely lacking in the modern diet, is the major source of insoluble fiber. Vegetables contain many vitamins, nutrients, and soluble fiber, which has numerous health benefits. Adding these foods to our diet after adding cellulose-digesting capabilities could help assuage the obesity epidemic and significantly improve human health. Improving human digestion could vastly reduce waste generated by humans and increase the efficiency of human consumption. We only need to better observe and understand those particular microbes to integrate them into our bodies, which are already structurally favorable for such a change. With the successful integration of microbes, we could cut down on food intake by making use of the cellulosic-energy, reduce cellulosic waste by turning it into food, solve problems of food shortages by making algae, grass, straw, and even wood edible and eventually turn human bodies into a source of renewable energy.

Reference: http://dujs.dartmouth.edu/2011/02/turning-waste-into-food-cellulose-digestion/

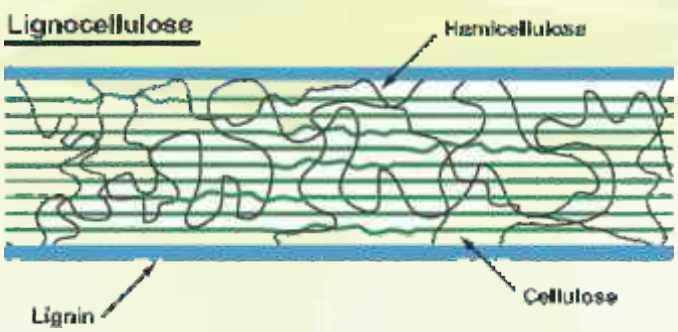
LIGNOCELLULOSE AS RAW MATERIAL IN FERMENTATION PROCESSES

Dr. Archana Prakash, Research & Development Department

INTRODUCTION

Lignocellulosic biomass comprising forestry, agricultural and agro-industrial wastes are abundant, renewable and inexpensive energy sources. Such wastes include a variety of materials such as sawdust, poplar trees, sugarcane bagasse, waste paper, brewer's spent grains, switchgrass, and straws, stems, stalks, leaves, husks, shells and peels from cereals like rice, wheat, corn, sorghum and barley, among others. Lignocellulose wastes are accumulated every year in large quantities, causing environmental problems. These materials are mainly composed of three groups of polymers, namely cellulose, hemicellulose, and lignin. Cellulose and hemicellulose are sugar rich fractions of interest for use in fermentation processes, since microorganisms may use the sugars for growth and production of value added compounds such as ethanol, food additives, organic acids, enzymes, and others. This is an overview about the potential uses of lignocellulosic materials in fermentation processes.

LIGNOCELLULOSE STRUCTURE



The major constituents of lignocellulose are cellulose, hemicellulose, and lignin, polymers that are closely associated with each other constituting the cellular complex of the vegetal biomass. Basically, cellulose forms a skeleton which is surrounded by hemicellulose and lignin.

TABLE: AVERAGE VALUES OF THE MAIN COMPONENTS OF LIGNOCELLULOSE WASTES

Lignocellulose waste	Cellulose weight (%)	Hemicellulose weight (%)	Lignin weight %
Barley straw	33.8	21.9	13.8
Corn cobs	33.7	31.9	6.1
Corn stalks	35	16.8	7.0
Oat straw	58.5	14.4	21.5
Rice straw	39.4	27.1	17.5
Rye Straw	36.2	19.0	9.9
Soya stalks	37.6	30.5	19.0
Sugarcane bagasse	34.5	24.8	19.8
Sunflower Stalks	40.5	27.0	10.0
Wheat stalks	42.1	29.7	13.4
Wheat straw	32.9	24.0	8.9

PRETREATMENTS FOR SELECTIVE SEPARATION OF LIGNOCELLULOSE CONSTITUENTS

Separation of cellulose, hemicellulose and lignin from lignocellulose biomass requires the use of specific processes, which may be physical, physico-chemical, chemical or biological because of differences in structures. The most commonly used lignocellulose hydrolysis are briefly described below.

1. **Chemical hydrolysis (concentrated acid hydrolysis)**
   Cellulose and hemicelluloses treated with mineral acids such as H2SO4 or HCl (in the range of 10– 30%), at temperatures of about 160ºC and pressures of about 10 atm. In the case of hemicellulases the use of diluted acids (1-4%), under moderate temperatures (120 to 160ºC), has proven to be adequate for hydrolysis, promoting little sugar decomposition.

2. **Enzymatic hydrolysis**
   Enzymatic hydrolysis has can be performed under milder reaction conditions (pH around 5 and temperature less than 50ºC) with lower energy consumption and lower environmental impact. In addition, it does not present corrosion problems, and gives high yield of pure glucose with low formation of by-products.

3. **Steam explosion hydrolysis**
   This method used for hemicelluloses in which biomass is heated using high pressure saturated steam (0.69-4.83 MPa, 160-260ºC) for a short period (from seconds to few minutes). Steam condenses under high pressure, thereby wetting the material, and then the pressure is suddenly reduced, which makes the material undergo an explosive decompression. Combination of acetic acid with sudden depressurization, promote the hemicellulose hydrolysis and solubilization. Limitations of steam explosion include an incomplete disruption of the lignin-carbohydrate matrix, and generation of compounds that may be inhibitory to microorganisms.

4. **Autohydrolysis**
   Autohydrolysis is a process similar to the steam explosion, but in this case, the explosion does not occur. This process uses compressed liquid hot water (≈ 200ºC; pressure > saturation point) and the acids resulting from hydrolysis of acetyl and uronic groups, originally present in hemicelluloses, catalyze hydrolysis of links between hemicelluloses and lignin as well as between the carbohydrates. This process is able to hydrolyze hemicellulose in minutes, with high yield, low by-products formation and no significant lignin solubilization.

5. **Alkaline and Organosolvents hydrolysis**
   Alkali treatments refers to the application of alkaline solutions such as NaOH, $Ca(OH)_2$ or ammonia. The alkali treatment causes swelling, leading to an increase in internal surface area, a decrease in the degree of polymerization, a decrease in crystallinity, separation of structural linkages between lignin and carbohydrates, and disruption of the lignin structure. Hydrogen peroxide treatment utilizes alkaline solutions at temperatures higher than 100ºC, which promote a fast decomposition of H_2O_2. As a consequence, more reactive radicals such as hydroxyl radicals (HO) and superoxide anions (O_2–) are produced, which are responsible for lignin degradation. Treatment with organosolvents involves the use of organic liquid like methanol, ethanol, acetone, ethylene glycol or triethylene glycol and water, with or without addition of catalysts such as oxalic, salicylic, and acetylsalicylic acid.

6. **Ozonolysis**
   Ozonolysis is used for lignin treatment in which lignin consumes most of ozone during the degradation of the carbohydrate content. As a consequence, low ozone amounts are available for cellulose degradation. Some advantages of this treatment are that ozonolysis are carried out at room temperature and normal pressure ozone can be easily decomposed.

7. **Biological treatments**
   Biological treatments, based on the use of brown, white and soft rot fungi have been commonly used to degrade the lignin, being considered a cheap and effective method of delignification. Degradation of lignin by fungi such as Phanerochaete chrysosporium, Trametes versicolor, Trametes hirsuta and Bjerkandera adusta, may be used to allow better access to the cellulose and hemicellulose components, besides to be also considered as an effective biological detoxification alternative.

8. **Submerged fermentation system (SmF)**
   Submerged fermentation systems can be defined as the cultivation of microorganisms in a liquid medium containing soluble carbon source and nutrients, maintained or not under agitation. Several characteristics make these systems attractive for the microorganisms cultivation and production of biological products, which include: i) the mixture of the medium is easy and allows uniform conditions for the microorganism growth; ii) modification of the cultivation conditions like pH, dissolved oxygen, temperature, agitation, and nutrient concentration are easy and fast; iii) the temperature control is favored by the high specific heat and thermal conductivity; iv) efficient technologies have already been developed, with high automation grade, diversity and availability of equipments and microbes.

9. **Solid state fermentation systems (SSF)**
   Solid state fermentation can be defined as any fermentation process allowing the growth of microorganisms on moist solid materials in the absence of free flowing water. The low moisture content means that fermentation can only be carried out by a limited number of microorganisms, mainly yeasts and fungi, although some bacteria have also been used. The nature of the solid substrate is one of the most important factor affecting SSF processes and its selection depends on several factors mainly related with cost and availability. Therefore, many studies have been performed involving the screening of several agro-industrial wastes as solid substrate in SSF.

CONCLUSION

Development of processes for reuse of forestry, agricultural and agro-industrial waste are of great interest. Since these wastes are rich in sugars, which are easily assimilated by microorganisms, they are very appropriate for use as raw materials in the production of industrially relevant compounds by fermentation. Products of interest for food, pharmaceutical and biofuels industries may be produced by fermentation of lignocellulose, both by submerged or solid state fermentation systems. Submerged fermentation systems require a previous step for fractionation of the lignocellulose to produce a sugar rich hydrolysate fermentable by microorganisms. Different treatments may be used for the lignocellulose fractionation and the selection of the best one must be done considering the product that will be produced and economical aspects. Solid-state fermentation systems are other interesting option to reuse lignocellulose and have as advantage the no need of raw material fractionation previous the use in the fermentation stage. Besides to serve as low cost raw materials for the production of important metabolites, the lignocellulose reuse in fermentation processes is an environment friendly method of waste management.

SUBMERGED FERMENTATION SYSTEM BASED PRODUCTS

Fermentation product	Agro-industrial solid substrate	Organism involved in fermentation
α-amylase	Rice straw, Rice bran, Red gram husk Jowar straw, Jowar spathe & Wheat bran	Bacillus subtilis, B. polymyxia, B. mesentericus, B. vulgarus, B. megaterium and B. licheniformis; however, Gibberella fujikuroi, Aspergillus oryzae
Cellulases	Sugarcane bagasse	Streptomyces sp.
Protease	Wheat bran, rice husk, rice bran & spent brewing grains, wheat bran	Aspergillus oryzae
Protease	Green gram, Chick pea, Red gram & Black gram husks and Wheat bran	Bacillus sp.
Fructosyl transferase	Corn cobs, Coffee husk, Spent coffee, Spent tea, Sugarcane bagasse, Cassava bagasse and Cereal brans	Aspergillus oryzae
Chitinase	Wheat bran–crude chitin mixture	Penicillium aculeatum
Tannase	Ber leaves, Jamun leaves, Amla leaves, Jawar leaves, Wheat bran, Rice bran, Sawdust & Sugarcane pith	Aspergillus sp.
Flavors (Fruity complex flavor)	Coffee husk	Ceratocystis fimbriata
Flavors (Banana flavor)	Cassava bagasse, wheat bran & sugarcane bagasse	Ceratocystis fimbriata
Flavors (Fruity complex flavor)	Cassava bagasse or giant palm bran	Kluyveromyces marxianus
Citric acid	sugarcane bagasse, Coffee husk and Cassava bagasse	Aspergillus niger
Gibberellic acid	Corn cobs	Giberella fujikuroi and Fusarium moniliforme
Tetracycline and Oxytetracycline	Corn cobs	Streptomyces rimosus
Ellagic acid	Pomegranate peel & Creosote bush leaves	Aspergillus niger
Destrucxins A & B (cyclodepsipeptides)	Rice husk	Metarhizium anisopliae

Source: S.I. Mussatto and J.A. Teixeira IBB–Institute for Biotechnology and Bioengineering, Centre of Biological Engineering

HOW TO MANAGE YOUR TIME EFFECTIVELY

Monika Choudhary, HR & Admin

"The two most powerful warriors are patience and time" ***Tolstoy***

WHAT IS TIME MANAGEMENT

"Time management" is the process of organizing and planning how to divide your time between specific activities. Good time management enables you to work smarter – not harder – so that you get more done in less time, even when time is tight and pressures are high. Failing to manage your time damages your effectiveness and causes stress.

It seems that there is never enough time in the day. But, since we all get the same 24 hours, why is it that some people achieve so much more with their time than others? The answer lies in good time management. The highest achievers manage their time exceptionally well. By using the time-management techniques in this section, you can improve your ability to function more effectively – even when time is tight and pressures are high.

Good time management requires an important shift in focus from activities to results: being busy isn't the same as being effective. (Ironically, the opposite is often closer to the truth). Spending your day in a frenzy of activity often achieves less, because you're dividing your attention between so many different tasks. Good time management lets you work smarter - not harder - so you get more done in less time.

The trick is to organize your tasks and use your time effectively to get more things done each day. This can help you to reduce stress and do better at workplace. Time management is a skill that takes time to develop and is different for each person. You just need to find what works best for you. Use few strategies listed below for few weeks and see if it can help you

10 WAYS THAT YOU CAN USE TO IMPROVE YOUR TIME MANAGEMENT SKILLS AND INCREASE PRODUCTIVITY

1. **Delegate Tasks**: It is common for all of us to take more tasks than our desired potential. This can often result in stress and burnout. Delegation is not running away from your responsibilities but is an important function of management. Learn the art of delegating work to your subordinates as per their skills and abilities.

2. **Prioritize Work:** Before the start of the day, make a list of tasks that need your immediate attention as unimportant tasks can consume much of your precious time. Some tasks need to be completed on that day only while other unimportant tasks could be carried forward to next day. In short, prioritize your tasks to focus on those that are more important.

3. **Avoid Procrastination:** Procrastination is one of the things that badly affect the productivity. It can result is wasting essential time and energy. It should be avoided at all costs. It could be a major problem in both your career and your personal life.

4. **Schedule Tasks:** Carry a planner or notebook with you and list all the tasks that come to your mind. Make a simple 'To Do' list before the start of the day, prioritize the tasks, and make sure that they are attainable. To better manage your time management skills, you may think of making 3 lists: work, home & personal.

5. **Avoid Stress:** Stress often occurs when we accept more work than our ability. The result is that our body starts feeling tired which can affect our productivity. Instead, delegate tasks to your juniors and make sure to leave some time for relaxation.

6. **Set up Deadlines:** When you have a task at hand, set a realistic deadline and stick to it. Try to set a deadline few days before the task so that you can complete all those tasks that may get in the way. Challenge yourself and meet the deadline. Reward yourself for meeting a difficult challenge.

7. **Avoid Multitasking:** Most of us feel that multitasking is an efficient way of getting things done but the truth is that we do better when we focus and concentrate on one thing. Multitasking hampers productivity and should be avoided to improve time management skills.

8. **Start Early:** Most of the successful men and women have one thing in common. They start their day early as it gives them time to sit, think, and plan their day. When you get up early, you are more calm, creative, and clear-headed. As the day progresses, your energy levels starts going down which affects your productivity and you don't perform as well.

9. **Take Some Breaks:** Whenever you find yourself for 10-15 minutes, take a break. Too much stress can take toll on your body and affect your productivity. Take a walk, listen to some music or do some quick stretches. The best idea is to take off from work and spend time with your friends and family.

10. **Learn to say No:** Politely refuse to accept additional tasks if you think that you're already overloaded with work. Take a look at your 'To Do' list before agreeing to take on extra work.

"Well done is better than well said." Benjamin Franklin or to paraphrase this in modern terms, "Don't talk the talk, instead walk the walk". Some people constantly talk about the things they are going to do but then keep procrastinating. Take action if you wish to get results.

A stitch in time would have confused Einstein.

If it weren't for time, everything would happen at once

STAI CONVENTION (July)

DSTA CONVENTION (August)

INDEPENDENCE DAY CELEBRATION

OFF SITE TRIP

welcome

NAME : Prakash Singh Bisht
DEPARTMENT : R&D Department
DATE of JOINING : July 1, 2016

NAME : Navin Tomar
DEPARTMENT : Accounts
DATE of JOINING : July 5, 2016

NAME : Neeraj Pathak
DEPARTMENT : Business Development
DATE of JOINING : August 19, 2016

NAME : Pardha Saradhi
DEPARTMENT : Customer Solutions
DATE of JOINING : August 22, 2016

NAME : Akash Gupta
DEPARTMENT : Supply Chain
DATE of JOINING : Sept 1, 2016

NAME : Subham Bhargava
DEPARTMENT : Accounts
DATE of JOINING : Sept 6, 2016

NAME : Namrata Tyagi
DEPARTMENT : R&D Department
DATE of JOINING : Sept 12, 2016

NAME : Neeraj Saini
DEPARTMENT : Supply Chain
DATE of JOINING : Sept 19, 2016

