

Review

A review of pentose utilizing bacteria from bagasse hemicellulose

Farhana Sharmin*, Flavia Huygens and Megan Hargreaves

Faculty of Health and School - Biomedical Sciences, Queensland University of Technology, Brisbane, GPO Box 2434, Qld 4001, Australia.

Accepted 17 May, 2013

There has been considerable increase in interest in the conversion of renewable resources into several essential products (for example, biofuel, amino acids, among others) over the last few years, as the potential impact of global warming is demanding technologies that close the carbon cycle. Utilization of the hemicellulose fraction from lignocellulosic biomass is an important factor to optimize for the economically feasible products. The bioconversion of pentoses derived from hemicellulose remains a bottleneck in developing industrial production, as microbes either preferentially use glucose, or cannot use pentoses at all. Sugar cane bagasse is one of the best candidates to have large amount of hemicelluloses for industrial interest. The aim of this review paper is to study research conducted on pentose utilizing bacteria, a variety of effects during break down of hemicellulose and the industrial importance of those bacteria.

Key words: Lignocelluloses, hemicellulose, pentose, xylose.

INTRODUCTION

Plant biomass is biodegradable and serves as a good alternative source of energy and chemical products because of its safety, reliability and resulting reduction of pollution. Rising costs, the finite nature of fossil fuels and the ecological problems associated with CO₂ emissions, are combining to create renewed interest in plant biomass as a sustainable basis for the production of alternative resources for energy, transport fuels and chemicals (Francesco Cherubini Energy Conversion and Management, 2010; Vishnu and Mala, 2012). Microbial utilization of lignocellulosic biomass for the production of commercially valuable products such as chemicals, liquid fuels, protein enriched food/feed, and preparation of cellulose polymers, is an attractive approach to help meet energy and food demands of developed and developing countries (Govindaswamy and Vane, 2007; Francesco Cherubini Energy Conversion and Management, 2010; Vishnu and Mala, 2012).

Lignocellulosic biomass contains several polymeric components such as lignin, cellulose and hemicellulose. Fractionation and enzymatic treatment can therefore yield

various product streams that are rich in phenolics from lignin, glucose from cellulose, and pentoses (mainly xylose and arabinose) from hemicellulose. Unfortunately, fermentation processes are involving a reaction of mixtures of sugars mixtures (glucose, xylose, arabinose and others) such as are present in the lignocellulosic biomass, usually results in the preferential use of glucose due to catabolite repression and consequent failure to fully utilize all of the available sugars. Although significant work has been carried out on pentose fermentation, no economically feasible process has been developed so far. Despite the natural pentose fermentation characteristics, yeast and fungi (Table 1) have several physiological drawbacks such as, long fermentation period, low productivity, high viscosity fermentation broth, and requirement of low critical oxygen levels and formation of by-products (ethanol, acetic acid, lactic acid) in large amounts during single fermented product accepted, inhibitors sensitivity (Dien et al., 2003; Hahn-Hägerdal et al., 2007; Gírio et al., 2010).

Commercial fermentation systems aim to maximize

*Corresponding author. Email: f.sharmin@qut.edu.au or khushi880@yahoo.com.

Table 1. Pentose utilizing microorganisms after genetic modification.

Bacteria	Fungi and yeast	Actinomycetes
<i>Z.mobilis</i>	<i>S. cerevisiae</i>	<i>Rhodococcus</i>
<i>Zymobacter palmae</i>	<i>Rhodotorula glutinis</i>	
<i>E. coli</i>	<i>Pichia stipitis</i>	
<i>Salmonella typhimurium</i>	<i>Candida shehatae</i>	
<i>Clostridium acetobutylicum</i>	<i>Aspergillus niger</i>	
<i>Bacillus subtilis</i>	<i>Rizopus solani</i>	
	<i>Fusarium oxysporum</i>	

Table 2. Comparison of various lignocellulosic raw materials.

Carbohydrate (% of sugar equivalent)	Corn stover	Wheat straw	Rice straw	Rice hulls	Bagasse fibre	Cotton gin trash	Newsprint	Populous tristis	Douglas fibre
Glucose	39.0	36.6	41.0	36.1	38.1	20.0	64.4	40.0	50.0
Mannose	0.3	0.8	1.8	3.0	n/a	2.1	16.6	8.0	12.0
Galactose	0.8	2.4	0.4	0.1	1.1	0.1	n/a	n/a	1.3
Xylose	14.8	19.2	14.8	14.0	23.3	4.6	4.6	13.0	3.4
Arabinose	3.2	2.4	4.5	2.6	2.5	2.3	0.5	2.0	1.1

Source: Hoch, 2007; Hahn-Hägerdal et al. 2007.

productivity, so that high product yields per unit of microbial biomass are produced, and the fullest possible use of the carbohydrate source provided, is always the preferred outcome. There are already a large number of research has done about cellulosic fermentation. Hexose sugar was the major utilizing substrate to utilization on those researches. However, there are very limited number of research has made about pentose sugar utilization. Additionally the reason of chosen sugar cane bagasse as a substrate because the amount of pentose sugars present in bagasse is higher than the pentose present in other sources (Table 2). As hemicelluloses contain large amount of pentose sugar, so the aim of this review to explore the microbial pentose fermentation.

HEMICEELULOSE AND SUGAR CANE BAGASSE: NATURE, SOURCES AND PRE-TREATMENT

Lignocellulosic materials, such as sugar cane bagasse, a waste product of the sugarcane processing industry (Womersley, 2006; Candido et al., 2012) may serve as an abundant and comparatively cheap feedstock for large scale industrial fermentation, resulting in the production of marketable end-products. However, the complex structure of lignocellulosic materials, the presence of various hexose and pentose sugars in the hemicelluloses component, and the presence of various compounds that inhibit the organisms selected for the fermentation process, all constitute barriers that add to the production costs and make full scale industrial production economically less feasible (Rainey, 2009).

Sugar cane bagasse is the fibrous matter that remains after sugarcane is crushed to extract the juice. A typical chemical analysis of bagasse is (on a washed and dried basis): cellulose 45–55%, hemicellulose 20–25%, lignin 18–24%, ash 1–4%, waxes <1% (Rainey 2009)[39]. Currently bagasse is being used as a primary fuel for sugar milling and processing operations, with occasional supplementation by sawdust, coal and fuel oil (Womersley, 2006; Candido et al., 2012). The component of bagasse that is of most interest in an industrial sense is the hemicellulose fraction due to its widespread availability and low cost. In contrast to cellulose, which contains only anhydrous glucose, sugar monomers in hemicellulose can include not only glucose, but also xylose, mannose, galactose, rhamnose, and arabinose.

Utilization of the hemicellulose fraction in lignocellulosic biomass is an important factor in optimizing the economics of biomass-related commercial processes given that hemicellulose makes up a significant proportion (44% carbon content) of the potentially available carbon for use in fermentation or chemical extraction processes (Hoch, 2007). Cellulosic biomass represents the only foreseeable, sustainable source of organic fuels, chemicals, and materials (Lynd et al., 2001; Hahn-Hagerdal et al., 2007). A primary technological challenge in biologically processing biomass into fuels and chemicals is that of overcoming the recalcitrance to hydrolysis. Although acid hydrolysis processes using acids are more technologically developed rather than, enzymatic processes. Enzymatic processes are comparable projected costs effective and are expected to enjoy an increasing cost advantage as the technology improves (Lynd et al., 2001;

Lynd et al., 2008; Jae-Han et al., 2010). Due to its resistance to enzymatic attack, biomass must be pre-treated before it can be enzymatically hydrolyzed.

To be economical, pretreatment process should minimize energy demands and limit costs associated with feedstock size reduction, materials of construction, and treatment of process residues (Lynd et al., 2008). Process conditions temperature, reaction time, pH, and biomass concentration affect these substrate factors, and thus influence pre-treatment performance. Pre-treatment processes can be of three categories: physical, chemical, and hydrothermal. Physical pre-treatments which are typical, demand large amounts of energy and are expensive, employing purely mechanical means to reduce feedstock particle size so as to increase available surface area. A variety of chemicals such as acids, alkalis, organic solvents, oxidizing agents and supercritical fluids have been considered for use as pre-treatment agents (Nathan-Mosier et al., 2005). Pre-treatment is one of the most expensive and least technologically mature unit operations in lignocellulosic conversion processes using enzymatic hydrolysis (Lynd et al., 2001, 2008). Hydrothermal pre-treatment refers to the use of water as liquid or vapour or both, to provide the heat to pretreat biomass. Relative to dilute acid pretreatment, hydrothermal pre-treatment processes have several potential advantages in particular the fact that there is no requirement for purchased acid, for special noncorrosive reactor materials or for preliminary feedstock size reduction (Nathan-Mosier et al., 2005). Furthermore, hydrothermal processes produce much lower quantities of hydrolyzate neutralization residues, which result from the process and may be an adverse influence on the formation of large amounts of biomass.

Different pre-treatment methods for preparing bagasse enzymatic hydrolysates have been investigated with focus on obtaining high sugar yields. The pre-treatment methods include steam explosion, liquid hot water pretreatment and pre-treatments with peracetic acid or with ammonia water. Martin et al. (2007) reported wet oxidation (WO) as a pre-treatment method for enhancing the enzymatic convertibility of sugarcane bagasse. Two types of reactions occur during WO, a low-temperature hydrolytic reaction and a high-temperature oxidative reaction. The studies by Martin et al. (2007) revealed that wet oxidation is an appropriate method for fractionating sugarcane bagasse and for enhancing its enzymatic hydrolysis. Alkaline WO at 195°C during 15 min gave the best results, yielding a solid material with nearly 70% biomass content with approximately 93% of hemicelluloses. A significant part of the polysaccharides was lost due to degradation and formation of by-products, mainly carboxylic acids, and the enzymatic convertibility of the pretreated material was poor. From the study, they found the tendency of WO to catalyze the transfer of hemicellulose from the solid phase to the liquid phase without a major hydrolysis of the solubilized hemicellulose mole-

cules. It was also reported that more xylose was formed by WO and more glucose formed more xylose by steam explosion (Martin et al., 2008; Lynd et al., 2008). It can be stated after the above discussion that sugarcane bagasse would be good substrate in fermentation technology in terms of pre-treatment procedure; there are more information about the bagasse in Table 1.

Pentose-fermenting microorganisms and potential products

A lack of microorganisms that are able to naturally and efficiently ferment hexoses and pentoses is a major constraint to the economic utilisation of biomass. Therefore, efforts have been made to obtain recombinant strains of bacteria and yeast able to meet the requirements of industrial lignocellulose fermentation. *Escherichia coli*, *Klebsiella oxytoca* and *Zymomonas mobilis* have all been genetically engineered to produce ethanol and biofuel efficiently from all hexose and pentose sugars present in the polymers of hemicellulose (Attfield and Philip, 2006; Hahn-Hagerdal et al., 2007; Kim et al., 2007; Yanase et al., 2007). Others mentioned in the literature included *Klebsiella* species, *Pichia stipitis*, *Bacillus* species and *Kluyveromyces* species. Conversely, those that are able to utilize pentose sugars, produce end-products at unacceptably low yields and productivity levels (Agbogbo et al., 2006; Ramesh et al., 2011). However, a number of drawbacks can also be identified:

1. The neutral preferred pH range (6–8) makes bacterial fermentation susceptible to contamination (Zhao et al., 2011).
2. The low tolerance to lignocellulose derived inhibitors (Hahn-Hägerdal et al., 2007)
3. Low ethanol tolerance (Kim et al., 2007).
4. Mixed product formation (ethanol, acetic acid, lactic acid and others) (Hahn-Hägerdal et al., 2007).
5. Reducing the yield of any single product such as ethanol (Hahn-Hägerdal et al., 2007).

Kawaguchi et al. (2006) and Miho et al. (2008) has reported that wild type *Corynebacterium glutamicum* was unable to utilize xylose under both standard aerobic and oxygen deprivation conditions, owing to the lack of xylose isomerase activity. However, metabolically engineered *C. glutamicum* has been widely used for the industrial production of various amino acids, nucleic acids and ethanol from sucrose and glucose based media. Miho et al. (2008) have attempted amino acid synthesis using *C. glutamicum*, which are using a xylose substrate and forcing carbon flow to ethanol production using high cell density fermentation under bacteriostatic conditions. This approach is unproven except in defined medium conditions and also relies on genetically engineered strains.

Some success has been achieved with natural selection, developing strains of *S. cerevisiae* that were able to utilize xylose (Govindaswamy and Vane, 2007; Akinori et al., 2009). While the growth rates achieved were quite low, the principle provides an interesting alternative to genetic engineering. Some limitations of genetic engineering methods include difficulties in using engineered microbes in large scale industrial processes, such as the possibility of back-mutation and the unsuitable physiological properties of the resulting strains, plus the problem of poor public acceptance. It was noted from the review that, both biocatalyst and genetically modified derivatives of microbes are potentially useful for the conversion of pentose-rich feed stocks such as corn stover, corn fiber, sugar cane bagasse, rice hulls, and rice straw into commodity chemicals such as lactic acid, fatty acids, acetic acid and several other industrial chemicals. However both processes are time consuming and expensive (Hahn-Hägerdal et al., 2007; Ramesh et al., 2011).

Natural pentose utilizing microorganisms

Several microorganisms, including certain bacteria, yeasts and filamentous fungi, have been reported as being able to ferment lignocellulosic hydrolysates (Table 1). Other bacterial strains capable of metabolising pentoses for the production of alternative fuels or industrial chemicals include: solventogenic *Clostridium sp.* for production of butanol from pentoses *Vibrio furnissii* capable of alkane production (Patel et al., 2006; Yu-Sin et al., 2012) and acid-tolerant, thermophilic *Bacillus* strains or *Lactobacillus pentosus* which can produce lactic acid (Gírio et al., 2010).

Bagasse is used only as a supplement to the sugars or starch hydrolyzate that comprised the major carbon source (Ítalo et al., 2011). The fungus *Rhizopus oryzae* and the bacterium *Lactobacillus* are reported to be useful for producing lactic acids from sugarcane bagasse (John et al., 2007). Generally, *Lactobacillus* species are deficient in cellulolytic and amylolytic capacity (that is, they lack the capability for breaking down starch into sugars), so necessitating the prior hydrolysis of cellulosic and starchy wastes to improve their utilization (John et al., 2007). Several end products such as (lactic acid, citric acid, acetic acid butanol, propionic acid etc) produced by the *Lactobacillus pentosus*, *Bacillus subtilis*, *Vibrio furnissii* and *Clostridium acetobutylicum* after pentose sugar utilization (Patel et al., 2006).

Metabolic Processes: inhibitors, media

Effects of metabolic inhibitors

Many of the dedicated energy crops can provide high energy biomass, which may be harvested several times each year (Lin and Tanaka, 2006). Even when suitable pentose-capable organisms, whether natural or genetically engineered are available, there remain difficulties of

process, which must be resolved. Some such difficulties encountered in this area of research will be now discussed.

The first issue to be resolved in studying application of the pentose fermentation pathways is an observed inhibition effect. Previous researcher reported that in order to minimize negative effects on the pentose fermentation process, it was necessary to: prevent accumulation of inhibitory components (such as furfurals, hydroxymethyl-furfural, acetic acid, among others) by detoxifying them, improve media composition, and allow the microorganism to acclimatize to the toxic inhibitors. A dilution step also decreases the necessity for addition of extra microbial nutrients and requires minimal changes to industrial fermentation plants and processes for its implementation (Chaabane et al., 2006).

A second issue related to the effective utilization of lignocellulosic biomass by fermentation is the presence of a mixture of carbon sources, the major components being cellulose, which is almost all glucose, and hemicellulose consists partly of pentose and partly of hexose sugars. Moreover, microorganisms generally use hexoses preferentially over pentoses. Therefore, reliance on the fermentation of pentoses alone could never become a viable strategy for commercial fermentation processes (Hahn-Hägerdal et al., 2006, 2007).

One very common outcome of mixed carbon source fermentation is the diauxy phenomenon, whereby one sugar (generally the energy efficient glucose) is used prior to the other(s). This characteristic can be easily observed by measuring the growth curves of an organism growing in a mixed carbon source medium, noting particularly that two distinct growth phases are present, separated by a lag period. The diauxy growth pattern is generally attributed to catabolite repression. The efficiency of sugar breakdown in microorganisms is dependent not only on the metabolic pathways available to the organism, but also on the effective transport of the sugar molecules into the microbial cell.

Unfractionated hydrolysates, including pentoses, would obviously be the preferred fermentation substrate so long as the microorganism can convert the various available sugars, whether they are pentoses or hexoses or a mixture of these. Culture-based solutions to the mixed carbon-sources problem include sequential fermentation with different microbial species, and the co-culture of several microorganisms with different substrate capabilities. Alternatively, the use of a single microorganism capable of using all of the substrates present is an attractive option.

Culture media

Among various cheap carbon sources, industrial by-products such as molasses and other cheaper components like wheat bran and sugar cane bagasse have been major focus, since they support both biomass increase, and enzyme production. The partial composition of culture

media as a natural sugarcane bagasse is xylose 25.2 and glucose 41.0, expressed as % w/w of the dry matter (Sharmin, 2012). Efficient use of hemicellulose fraction as a fermentation feed-stock would thus require the use of microbes that can metabolise such pentoses in the presence of glucose, resulting in efficient use of the sugars available.

Growth conditions such as pH; availability of suitable levels of nutrients such as nitrogen, phosphorus and trace elements; optimum temperature range, oxygen availability during growth and depletion during fermentation all must be optimized before fermentation. In particular, the pH should be at the optimum level during growth, but during end-product production the pH may be reduced (Haq et al., 2003; Berovic et al., 2007).

Industrial applications and future recommendations

Industrial fermentation of bagasse hemicellulose depends on resolution of several issues, the first being determination of the identity, nature, and relative size of populations of the different bacteria present in bagasse leachate and bulk material. This is necessary in order to explore the possibility of a dynamic relationship during the industrial process. Further information would be available by measuring their presence and numbers at various, specific stages of the degradation process (Patel et al., 2004; Miho et al., 2008).

One option for hemicellulose fermentation requires microbes that can metabolise the pentoses in the presence of glucose, preferably without being subjected to catabolite repression and so being capable of the simultaneous use of the sugars. A second possibility is that the selected microorganism should carry out an efficient diauxy process using two or more sugars sequentially. A third option is to use a sequential process involving a number of microorganisms, each performing fermentation processes, thus permitting the selective fermentation of hemicellulose pentoses within a mixture of substrates. In such a scenario, available glucose could be used in a separate fermentation process (Attfield and Philip, 2006). This is an attractive option, because large scale ethanol production by *S. cerevisiae* is an established technology that will be hard to replace by alternative biological processes (Hahn-Hägerdal et al., 2006, 2007). However this can be an alternation for bacteria.

While industrial biofermentation of sucrose and other hexose substrates has been studied extensively, pentose sugars substrates have not been analyzed in such detail. In order to achieve industrially cost-effective fermentation processes on pentose-containing feed-stock, conditions will have to be adjusted to permit greatest biomass production, and so high product yield. Trials will be required to determine the optimum temperatures for biomass production and product yield, ideal pH levels, ideal nutritional composition and incubation periods for maximum bio-

mass formation and feed-stock degradation. In addition, enzymatic analysis in order to understand the activity of enzymes responsible for the degradation of pentoses, will permit study of the possibility of up-regulation. Finally, quantitative analysis of substrate sugars consumed and end-products produced will aid in measurement of the efficiency of the processes.

Bagasse leachate can be used as a substrate, however before fermentation starts, the bagasse may need to be treated to destroy the unwanted wild microorganisms present. If using heat to treat the bagasse leachate, the sugars present will be caramelized, and the carbon could be rendered unsuitable for use by microorganisms. Steam fractionation could be an alternative. Timing is a main issue for steam fractionation. Steam fractionation of bagasse leachate could be an expensive process for the scaling up of end-product formation. According to Miho et al. (2008) bagasse samples were extracted as a water-soluble or steam fraction at low temperatures (200–230°C), and about 30% was extracted at higher temperatures (230–280°C). At 200–230°C, hydrolysates of hemicellulose (galactose, arabinose and xylose) and aromatic compounds mainly existed in the extract solution.

Another necessary step is the measurement of bacterial cell concentrations during biomass production. Industrial fermentation uses fed-batch cultivation of single-celled organisms, occurring firstly with and then without, the presence of air. In most industrial processes, the biomass is generated under aerobic conditions, followed by the anaerobic fermentation step, during which the end-products are generated. Fed-batch fermentation is an Industrial fermentation technique that falls between batch and continuous fermentation (Hewitt and Nienow, 2010). A controlled feed rate, with the right component constitution is required during the process. Fed-batch offers many advantages over batch and continuous cultures. From the concept of its implementation, it can be easily concluded that under controllable conditions and with the required knowledge of the microorganism involved in the fermentation, the feed of the required components for growth and/or other substrates required for the production of the product can never be depleted and the nutritional environment can be maintained approximately constant during the course of the batch.

Sometimes, controlling the substrate is also important due to catabolic repression. Since this method usually permits the extension of the operating time, high cell concentrations can be achieved and thereby, improved productivity (mass of product/volume x time). This aspect is greatly favored in the production of growth-associated products (Hewitt and Nienow, 2010).

Another advantage of fed-batch fermentation is that it requires no specialized equipment, while the batch fermentation requires sterilization operating system to prevent contamination. However, a large scale fermentation process of any type requires “specialized” equipment consisting of fermentation vats of suitable size. An addi-

tional advantage of cyclic fed batch cultivation is the extension of productive phase under controlled conditions. The controlled periodic shifts in growth rate provide a good chance of optimization of secondary metabolite synthesis during the deceleration in growth (Hewitt and Nienow 2010; Sharmin 2012).

Following the creation of biomass, the process is changed to an anoxic state by stopping the bubbler to allow microbes to produce fermentation products. Shaking should continue, to prevent the biomass from precipitating to the floor of the vessel. The process is not unlike that of beer production, in which, following aerobic biomass production, the yeast is stirred slightly in order to remain fully distributed through the beer during the anaerobic fermentation step (McPhee, 2003).

As discussed above, different carbon sources as growth supplements should be tested, in order to explore the possibility of different end-products. This review has examined currently available literature regarding the Industrial possibilities of microbial fermentation of plant-processing wastes such as sugarcane bagasse. It can be concluded that bioconversion of bagasse could be economically advantageous for the production of enzymes, animal feed, bioethanol and bioplastics. Since untreated bagasse is degraded very slowly by micro-organisms, a pre-treatment step may be useful for improved substrate utilization. Similarly, although many efforts have been made on sugarcane bagasse hydrolysis using pre-treatment methods as well as enzymatic saccharification, research needs further inputs into the process is increasing, and it may be useful as waste material from this has great potential for the future in terms of research and development.

ACKNOWLEDGEMENTS

We wish to acknowledge Prof. Margaret Britz for giving us some initial idea. Also thanks to Queensland University of Technology, Brisbane, Australia to giving us for the research opportunity.

REFERENCES

- Agbogbo FK, Kelly-Coward G, Torry-Smith M, Wenger KS (2006). Fermentation of glucose/xylose mixtures using *Pichia stipitis*. *Process Biochem.* 41: 2333-2336.
- Akinori M, Hiroyuki I, Tsutomu K and Shigeki S (2009). Ethanol production from xylose in engineered *Saccharomyces cerevisiae* strains: current state and perspectives. *Appl. Microbiol. Biotechnol.* 84(1): 37-53.
- Attfield PV, Philip JLB (2006). Use of population genetics to derive non recombinant *Saccharomyces cerevisiae* strains that grow using xylose as a sole carbon source. *FEMS Yeast Res.* 6(6):862-868.
- Berovic ML, Matic M, Raafat EG (2007). Citric acid production. *Biotechnol. Annu. Rev.* 13: 303-343.
- Candido RG, Godoy GG, Gonçalves AR (2012). Study of Sugarcane Bagasse Pre-treatment with Sulfuric Acid as a Step of Cellulose Obtaining. *WASET* 61: 101-105.
- Chaabane FB, Aldigui SA, Alfenore S, Cameleyre X, Blanc P, Bideaux C, Guillouet ES, Roux G, Molina-Jouve C (2006). Very high ethanol productivity in an innovative continuous two-stage bioreactor with cell recycles. *Bioprocess Biosyst. Eng.* 29(1):49-57.
- Francesco Cherubini Energy Conversion and Management (2010). The biorefinery concept: Using biomass instead of oil for producing energy and chemicals. 51:1412-1421
- Girio MF, Fonseca C, Carvalheiro F, Duarte LC, Marques S and Bogel-Lukasik R (2010). Hemicelluloses for fuel ethanol: A review. *Bioresour. Technol.* 101(13): 4775-4800.
- Govindaswamy S, Vane LM (2007). Kinetics of growth and ethanol production on different carbon substrates using genetically engineered xylose-fermenting yeast. *Bioresour. Technol.* 98 (3): 677-685.
- Hahn-Hägerdal B, Galbe M, Gorwa-Grauslund MF, Lidén G, Zacchi G (2006). Bio-ethanol - the fuel of tomorrow from the residues of today. *Trends Biotechnol.* 24 (12): 549-556.
- Hahn-Hägerdal B, Karhumaa K, Fonseca C, Spencer-Martins I, Gorwa-Grauslund M (2007). Towards industrial pentose-fermenting yeast strains. *Appl. Microbiol. Biot.* 74 (5):937-953.
- Haq I-U, Ali S and Iqbal J (2003). Direct production of citric acid from raw starch by *Aspergillus niger*. *Process Biochem.* 38 (6):921-924.
- Hewitt CJ, Nienow AW (2010). The scale-up of microbial batch and fed-batch fermentation processes. In: Marin Berovic M and Enfors S (eds). *Comprehensive Bioprocess Engineering*. Ljubljana: University of Ljubljana. pp. 295-320.
- Hoch G (2007). Cell wall hemicelluloses as mobile carbon stores in non-reproductive plant tissues. *Funct. Ecol.* 21 (5):823-834.
- Ítalo TSRM, Luciana AC-B, Pedro QCN, Spartaco AF (2011). Cultivation of *Trichosporon mycotoxinivorans* in sugarcane bagasse hemicellulosic hydrolyzate. *Electron. J. Biotechnol.* 15(11).
- Jae-Han K, David EB, David AM (2010). Simultaneous consumption of pentose and hexose sugars: an optimal microbial phenotype for efficient fermentation of lignocellulosic biomass. *Appl. Microbiol. Biotechnol.* 88(5):1077-1085.
- John R, Nampoothiri K, Pandey A (2007). Fermentative production of lactic acid from biomass: An overview on process developments and future perspectives. *Appl. Microbiol. Biot.* 74 (3): 524-534.
- Kawaguchi H, Vertes AA, Okino S, Inui M, Yukawa H (2006). Engineering of a xylose metabolic pathway in *Corynebacterium glutamicum*. *Appl. Environ. Microbiol.* 72 (5):3418-3428.
- Lin Y, Tanaka S (2006). Ethanol fermentation from biomass resources: Current state and prospects. *Appl. Microbiol. Biot.* 69 (6):627-642.
- Lynd LR, Laser MS, Bransby D, Dale BE, Davison B, Hamilton R, Himmel M, Keller M, McMillan JD, Sheehan J, Wyman CE (2008). How biotech can transform biofuels. *Nat. Biotechnol.* 26:169-172.
- Lynd LR, South LK, Van CR, Walsum GP, Levenson K (2001). Evaluation of paper sludges for amenability to enzymatic hydrolysis and conversion to ethanol. *Tippis J.* 84 (2):50.
- Martin C, Helene BK, Anne BT (2007). Wet oxidation as a pre-treatment method for enhancing the enzymatic convertibility of sugarcane bagasse. *Enzyme Microb. Technol.* 40:426-432.
- Martin C, Marcet M, Anne BT (2008). Comparison between wet oxidation and steam explosion as pre-treatment method for enzymatic hydrolysis of sugarcane bagasse. *Bio. Resour.* 3:670-683.
- McPhee J (2003). The unacknowledged workers of the biotechnology industry: Microbes. In *The Science Creative Quarterly*, British Columbia, Canada.
- Miho S, Toru J, Masayuki I and Hideaki Y (2008). Simultaneous utilization of D -cellobiose, D -glucose, and D -xylose by recombinant *Corynebacterium glutamicum* under oxygen-deprived conditions. *Appl. Microbiol. Biotechnol.* 81: 4
- Nathan-Mosier CW, Dale B, Elander R, Lee Y, Holtzapple M and Ladisch M (2005). Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresour. Technol.* 96 (6):673 (6).
- Patel M, Ou M, Ingram LO, Shanmugam KT (2004). Fermentation of sugar cane bagasse hemicellulose hydrolysate to l(+)-lactic acid by a thermotolerant acidophilic *Bacillus* sp. *Biotechnol Lett.* 26 (11):865-868.
- Patel MA, Ou MS, Harbrucker R, Aldrich HC, Buszko ML, Ingram LO, Shanmugam KT (2006). Isolation and characterization of acid-tolerant, thermophilic bacteria for effective fermentation of biomass-

- derived sugars to lactic acid. *Appl. Environ. Microbiol.* 72 (5):3228-3235.
- Rainey (2009). A study into the permeability and compressibility properties of bagasse pulp. PhD thesis, Queensland University of Technology, Brisbane, Australia.
- Ramesh CK, Rishi G, Yogender PK, Ajay S, Zhang YHP (2011). Bioethanol production from pentose sugars: Current status and future prospects. *Renewable Sustain. Energy Rev.* 15(9):4950–4962.
- Sharmin F (2012). Metabolic and ecological study of environmental pentose utilizing bacteria (E-PUB). PhD Thesis. Queensland University of Technology, Brisbane, Australia.
- Vishnu M, Mala R (2012). Trends in bioconversion of lignocellulose: Biofuels, platform chemicals and biorefinery concept. *Prog. Energy Combust. Sci.* 38(4):522–550.
- Womersley J (2006). Guideline: Managing impacts from the bulk storage of bagasse, Department of environment and resource management publication, Queensland, Australia.
- Yanase H, Sato D, Yamamoto K, Matsuda S, Yamamoto S, Okamoto K (2007). Genetic engineering of *Zymobacter palmae* for production of ethanol from xylose. *Appl. Environ. Microbiol.* 73 (8):2592-2599.
- Yu-Sin J, Joungmin L, Alok M, Do YS, Jung HC, Sang YL (2012). Butanol production from renewable biomass: Rediscovery of metabolic pathways and metabolic engineering. *Biotechnol. J.* 7: 186–198.
- Zhao G-Z, Li J, Zhu W-Y, Klenk H-P, Xu L-H, Li W-J (2011). *Nocardia artemisiae* sp. Nov. an endophytic actinobacterium isolated from surface-sterilized stem of artemisia annual. *Int. J. Syst. Evol. Micro.* 61(1):2933-2937.