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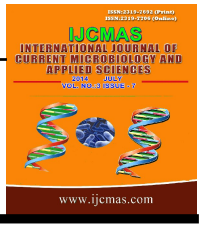

Review Article

Cactus pear biomass, a potential lignocellulose raw material for Single Cell Protein production (SCP): A Review

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A B S T R A C T

Global population increase in the past few decades has intensified protein malnutrition especially in the developing world where agriculture industry is not reputable. Promising biotechnological methods has been established to alleviate the world's protein deficit from ailing conventional protein sources. Single cell protein production (SCP) from lignocellulose biomass presents upcoming technology aimed at providing protein supplement for both human food and animal feeds. Microorganisms such as algae, fungi, yeast and bacteria, are involved in bioconversion of low-cost carbon feed-stocks such as lignocellulose to produce biomass rich in proteins and amino acids. Different lignocellulosic biomass are treated using chemical and biological methods to produce SCP from the microorganisms. The cladodes of *Opuntia ficus-indica* (cactus pear) is one such lignocellulosic raw material that has a potential for production of SCP in arid and semi-arid regions. This article highlights the current uses of lignocellulosic biomass and the use of cactus pear biomass as potential raw material in SCP production.

Introduction

The world's human population has increased by about 250% since the 1950s to the present, with an increase from 2.6 to 7 billion people and it is projected to grow from 7 to 9 billion people by 2042 according to the US Census Bureau. Some experts are uncertain whether the earth can support the present human population, let alone another 2 to 3 billion people (Holechek, 2007; Pimentel *et al.*, 1994). Their concern centres around the heavy dependency on fossil fuels and the impact of climate change on food production. During the past decades agricultural production has risen in most developed countries. However, most developing countries are still suffering from hunger, malnutrition, food insecurity and food related diseases. The World Health Organization (WHO) estimates that 12 million people die of hunger and starvation related diseases every year, half of whom are children under the age of 5 years (Israelidis, 2008).

Livestock and meat products have been among the fastest growing components of the global agriculture and food industry. This growth reflects not only increasing demand for meat as global incomes have risen, but also improved efficiencies in production and processing. This so-called livestock revolution has been an important feature of both developed and developing countries. Dependency on agriculture for the production of food and livestock feed has increased in recent years due to the increasing human population, a higher demand for protein food of animal origin and the increasing demand for carbon dioxide neutral biomass for the production of biofuels (Olstorpe *et al.*, 2008). Protein rich products of animal origin form an important part of the human diet and there are pressing needs to enhance livestock and poultry production to expand meat, milk and egg supplies to meet the need of the ever growing human population. In Africa, most livestock subsists on the available feeds, which are natural grasslands and several low quality crop residues such as cereal straws, but the grasslands are deteriorating due to overgrazing (Ben Salem *et al.*, 2002). Imbalanced feeding and poor health management of livestock are the main factors responsible for the animals' general low productivity.

In South Africa, natural pastures are the most important agricultural resources that provide the cheapest source of animal feed (Aucamp & Danckwerts, 1989). However, natural grazing is threatened by expansion of the Karoo (semi-desert region) into grasslands, overgrazing, poisonous plants, poor grazing management practices, bush densification and human activities such as agriculture, housing and industrialization. All these place a demand on the already overburdened natural grazing resources (Malherbe & Cloete, 2002). The production of fodder in the semi-arid region is restricted by the irregular distribution of rain, resulting in low availability of livestock feed during the drought season. This results in the periodic use of commercial protein sources as supplements in feeds. Due to the recurrent price rise of the commercial protein supplements used in feeding the animals, supplementation is becoming a non-economical activity for the breeders (Araújo *et al.*, 2005). Therefore, the substitution of single cell protein (SCP) for the commercial protein supplements, namely the fishmeal and soybean meal, has attracted considerable interest in the dairy and poultry industries (Chanda & Chakrabarti, 1996; Rajoka, 2005).

Single cell protein production usually involves the bioconversion of low-cost carbon feedstocks or low value by-products and wastes into value added microbial biomass. The economics of the process is mainly determined by the cost and availability of the carbon feedstock. Lignocellulosic biomass presents a readily available feedstock for microbial bioconversion which does not compete with feedstocks used for human food. Lignocellulose is the major structural component of woody plants and non-woody plants such as grasses and represents a major source of renewable organic matter. Lignocellulosic biomass is rich in fermentable sugars, high in fibre but low in protein content. The cladodes of *Opuntia ficus-indica*, the prickly pear cactus, is one example of such lignocellulosic biomass. *O. ficus-indica* is well adapted for cultivation in semi-arid regions, with a yield of 10 to 40 tonnes (dry weight) cladode biomass per ha. The cladodes of the spineless *O. ficus-indica* cultivars are suitable as livestock feed during periods of drought. However, because of their low crude protein content

(Malainine *et al.*, 2003; Stintzing & Carle, 2005), supplementation with other protein sources is desirable for use as a balanced animal feed ration. The cost of supplementation of the cladodes with fishmeal or soybean meal would be prohibitively expensive, however.

Bioconversion of cactus pear cladodes into SCP is a novel, productive and economic way of utilising the waste raw material than conventional means. This review focuses on composition, treatment and bioconversion of lignocellulosic raw material into SCP using different microorganisms. The use of cactus pear cladodes as a potential biomass for SCP was also reviewed.

Materials and Methods

Single cell protein

Single cell protein as dietary supplement for human and animal nutrition

Food products containing microorganisms have been included in human diets, either deliberately or accidentally, for thousands of years, although the consumption of microbial biomass is not widely accepted by some people. Microbial biomass is responsible for the production of food products such as alcoholic beverages, cheese, bread, yogurt and soya sauce (Tuse, 1984). Humans have enjoyed the delightful taste of basidiomycete fungi (mushrooms) and a few ascomycetes (yeasts) for thousands of years. The term single cell protein (SCP) was first introduced in 1968 at a meeting held at the Massachusetts Institute of Technology (MIT) to give a better image than "microbial protein" and "petroprotein", which were the terms originally used (Anupama & Ravindra, 2000; Mateles *et al.*, 1967).

The first purposeful SCP production originated in Germany during World War I, with the cultivation of bakers' yeast, *Saccharomyces cerevisiae*, on molasses and ammonium salts to serve as a protein supplement to replace as much as 60% of the foodstuffs Germany had been importing prior to the war (Boze *et al.*, 1992; Giec & Skupin, 1988; Litchfield, 1983). Later, during World War II, *Candida utilis* was cultivated on diverse waste products from the paper industry to serve as a protein source for both humans and animals (Litchfield, 1979). After World War II, cultivation of fungi in submerged culture for the production of antibiotics led to an investigation of the potential of microfungi as flavour additives to replace mushrooms. In the 1950s some oil industries became interested in the growth of microorganisms on alkanes as a feedstock for SCP production for feed and food (Litchfield, 1980).

Cellular composition of yeasts, filamentous fungi, bacteria and algae

The impressive advantages of microorganisms for single cell protein production compared to conventional sources of protein (soybeans or meat) are well known. These are cultured on abundantly available agricultural and industrial wastes (Robinson & Nigam, 2003; Singh *et al.*, 1988; Villa Bôas *et al.*, 2002). The four microbial groups that are used for SCP production are yeasts, filamentous fungi, bacteria and algae. The average biomass composition of these main groups is shown in Table 1 and their amino acid profile in Table 2.

Microorganisms have the ability to upgrade low protein plant material to high protein feed because they have a high protein content and a high growth rate, leading to rapid biomass production that is independent of the climatic conditions (Bekatorou *et al.*,

2006). The three main criteria for the selection of a particular microorganism for successful SCP production are (a) the nature of the raw material available, (b) its nutritional composition: amino acid profile, energy value, balanced protein and lipid content, vitamins and palatability, and (c) its toxicological composition: nucleic acid content, allergies and gastrointestinal effects. The ideal microorganism should possess the following technological characteristics: (a) a high specific growth rate and biomass yield, (b) a high affinity for the substrate with a low requirement for growth factor supplementation, (c) the ability to develop a high cell density, (d) a capacity for genetic modification and (e) a tolerance to temperature and pH changes (Boze *et al.*, 1992; Lichtfield, 1983).

Yeasts have been involved in human food for thousands of years, namely for brewing beer, fermenting wine and baking bread. Yeasts are rarely toxic or pathogenic, with most attaining the generally regarded as safe (GRAS) status and can be used in human diets. Thus, yeasts are more readily accepted as a food and feed supplement than are the other microbial groups. Although their protein content rarely exceeds 60%, the concentration of essential amino acids such as lysine (6 – 9%), tryptophan (1 – 1.5%) and threonine (4 – 6%) is satisfactory. By contrast, they contain only small amounts of the sulphur-containing amino acids, methionine and cysteine (Bekatorou *et al.*, 2006; Boze *et al.*, 1992).

They are rich in B group vitamins and their nucleic acid content ranges from 4 to 10%. Yeasts are larger in size than bacteria and this facilitates the separation of yeasts from the culture broth. Yeast such as *Kluyveromyces* spp, *Candida* spp and *Saccharomyces cerevisiae* have been successfully used in SCP production (Bhalla & Joshi, 1994; Ejiofor *et al.*, 1996; Pandey *et al.*, 2000; Rajoka *et al.*, 2006; Schultz *et al.*, 2006; Yakoub Khan *et al.*, 1992).

Bacteria generally have a specific growth rate and biomass yield greater than those of the other microbial groups, with a higher crude protein content of up to 80%. Their amino acid profile is balanced and their lysine content is high. Bacteria possess the ability to utilize a wide range of substrates, some of which are not metabolized by yeasts. However, a number of bacterial species are pathogenic and have higher nucleic acid content than yeasts, which is detrimental to human consumption. In addition, separation is difficult because of the relatively small size of bacteria (Boze *et al.*, 1992).

Filamentous fungi have specific growth rates and biomass yields that are lower than those of bacteria and yeasts. Their protein content is often less and they are also deficient in sulphur-containing amino acids. The fungal cell wall is rigid and there are problems of wall digestibility. However, their nucleic acid content is low.

The principal merits of filamentous fungi are their ability to use a large number of complex growth substances such as cellulose and starch and easy recovery by simple filtration, reducing production costs (Boze *et al.*, 1992). The most successful single cell protein product from filamentous fungi accepted for human consumption is *Fusarium venenatum* (formerlly *Fusarium graminearum*) grown in continuous culture to produce a mycoprotein product that is sold under the trade name Quorn^{TM} in the UK, the USA and at least eight other European countries, including Belgium, Denmark, France, Germany, Ireland, the Netherlands, Sweden and Switzerland (Wiebe, 2004).

The potential merits of algae are related to their ability to grow with CO_2 as the only carbon source. Algal production takes place mostly in natural water bodies such as ponds, lakes and lagoons. Algal production in ponds (open systems) with control of contamination by using highly alkaline or saline selective environments or by using fully closed photobioreactors (closed systems) are in operation (Miron *et al.*, 2003).

Algae are traditionally a food complement for some populations in Mexico (*Spirulina platensis*) and Chad (*Spirulina maxima*). Algae have a low sulphur-containing amino acid content and a low nucleic acid content. Algal cells are easy to recover, but their growth rate is slow and the investment costs in respect of a photobioreactor are greater than for a conventional bioreactor (Boze *et al.*, 1992).

Although microorganisms are grown primarily for their protein content, microbial cells also contain carbohydrates, lipids, vitamins, minerals and non-protein nitrogenous material such as nucleic acids. The protein and lipid content are determined to some extent by the composition of the culture medium and growth conditions. Filamentous fungi and yeasts have higher lipid and lower protein contents when grown on media rich in carbon substrate and poor in nitrogen.

Apparently microbial growth conditions have a limited influence on the amino acid content of SCP, including the deficiency of sulphur-containing amino acids (Giec & Skupin, 1988). A major advantage of SCP is its high lysine content, whereas plant protein is generally low in lysine. Thus, it is advantageous to use SCP supplements together with plant biomass in livestock feed products.

Substrates for single cell protein production

Conventional substrates such as starch, molasses, distiller's wash, whey, fruit and vegetable wastes have been used for SCP production, as well as unconventional ones such as petroleum by-products, natural gas, ethanol, methanol and lignocellulosic biomass (Bekatorou *et al.*, 2006; Martin, 1991). Carbohydrate substrates are the most widely used for SCP production due to the fact that carbohydrates are natural microbial substrates and also because carbohydrates constitute a renewable feedstock (Ugalde & Castrillo, 2002). The availability of the substrate and its proximity to the production plant are the major factors that determine the design and strategy of an SCP production process.

Molasses, the residual liquid obtained after crystallization of sugar from the concentrated sugar solution obtained from the milling of sugar cane or sugar beet, contains 45–55 % sugars, namely sucrose, glucose, fructose, raffinose, melibiose and galactose. It is estimated that for every 100 kg of cane milled for sugar production, some 3.5 to 4.5 kg of molasses is obtained (Oura, 1983). The use of molasses for the production of SCP is determined by its availability and low cost, its composition and absence of toxic substances and fermentation inhibitors (Bekatorou *et al.*, 2006). Though molasses is a suitable carbon feedstock for SCP production, it requires supplementation with ammonia salts and phosphorus salts (Ugalde & Castrillo, 2002).

Starch may be derived from rice, maize and other cereals. Cassava is a tropical root crop produced in more than 80 countries and it is a rich source of starch for SCP production (Ejiofor *et al.*, 1996).

In Sweden, the 'Symba" process was developed in the 1960s where starchy waste was utilised by combining two yeast species in a mixed culture (Jarl, 1969; Ugalde & Castrillo, 2002). The yeast mixed culture consisted of the amylase producing *Saccharomycopsis fibuligera* and the fast growing *Candida utilis*. Quorn™ mycoprotein from *Fusarium venenatum* is currently grown on glucose obtained from maize, but it was reported earlier that the process used wheat starch, a by-product from wheat gluten production (Trinci, 1994).

Another likely carbon substrate is cheese whey, the residual liquid obtained after the removal of protein and fat from milk during the curding process in cheese production or after ultra-filtration procedures for the production of spreading cheeses. Whey is produced worldwide in large amounts and its disposal causes serious environmental problems due to its high organic load, having a COD of 35 000–68 000 mg l^{-1}. About 46 million tonnes of cheese whey are produced annually in Europe alone (Schultz *et al.*, 2006). On the other hand, whey has a significant nutritional value since it contains substantial amounts of proteins, lactose, organic acids, fat, vitamins and minerals. Whey was used for the production of SCP using the lactose assimilating yeast strains *Kluyveromyces lactis, K. marxianus* and *Candida pseudotropicalis*, but only the *Kluyveromces* species were used for biomass production from whey on a commercial scale (Bekatorou *et al.*, 2006; Schultz *et al.*, 2006).

Alkanes were used as substrate for SCP production on a large scale in the former Soviet Union. A number of microorganisms can assimilate *n*-alkanes in liquid culture, including certain yeasts and filamentous fungi. However, SCP production from hydrocarbons presents practical complications due to the low water solubility of the substrate, as well as the high degree of aeration required for its utilisation (Smith, 1980). The major demerit of SCP production from alkanes is the toxicity of the substrate. Currently, SCP production from alkanes is not in operation. Methanol, a by-product of the petrochemical industry, has been used as a substrate for SCP production. The main advantage of methanol over other hydrocarbons is the volatile nature of the substrate, allowing for easy evaporation in the microbial biomass drying process (Ugalde & Castrillo, 2002).

Cellulose is the major constituent of all plant materials, which forms about half to one-third of plant tissues and is constantly replenished by photosynthesis. Agricultural residues such as corn stover, wheat straw, bagasse, plant residues and municipal solids are waste materials or low value feedstocks with enormous potential for SCP production (Gonzalez-Valdes & Moo-Young, 1981; Moo-Young & Chisti, 1994). The productivity of forests and woodlands amounts to 40% of the world net productivity, whereas the productivity from cultivated land amounts to a mere 6% (Ugalde & Castrillo, 2002). A list of various microorganisms and the carbon and energy sources they are able to utilise is given in Table 3.

In the recent past, the large-scale use of starch, agricultural wastes and molasses-based carbon substrates have proven economically viable for SCP production for animal feed and human consumption (Paul *et al.*, 2002). However, it has been demonstrated that the production of SCP from hydrocarbons was not economically competitive compared with other protein sources such as soybean, peanut and cottonseed flours due to high prices of crude

oil (Dìaz Ricci *et al.*, 1987; Moo-Young *et al.*, 1977).

Lignocellulosic biomass as raw material for SCP

Lignocellulose

Lignocelluloses serve as the major structural component of all plant biomass and represent the major source of renewable organic matter, making it a substrate of enormous biotechnological importance (Malherbe & Cloete, 2002). Lignocelluloses are either derived as a by-product from agricultural products or can be derived from plant biomass grown on non-agricultural or marginal lands, ultimately for conversion to fuels, feeds and chemicals (Howard *et al.*, 2003). The nature and availability of lignocellulosic feedstocks in different parts of the world depends on climate and other environmental factors, agricultural practice and technological development. Lignocelluloses are composed of various biopolymers, sugars and chemicals which could be of commercial value. Unfortunately, most lignocelluloses are disposed of as waste. Lignocellulosic feedstocks that have attracted attention for research on SCP production include corn stover, apple pomace, sugarcane bagasse, rice polishings, rice husks, maize cobs, maize fibre and citrus waste (Bhalla & Joshi, 1994; Pandey *et al.*, 2000; Rajoka *et al.*, 2006; Robinson & Nigam, 2003; Singh *et al.*, 1988; Villa Bôas *et al.*, 2002; Yakoub Khan *et al.*, 1992; Zhang *et al.*, 2006). Production of SCP from lignocelluloses is gaining much attention, with the recovery of valuable by-products and simultaneous reduction of the organic load as the chief economic advantages of such processes. Bioconversion of lignocelluloses for SCP production requires various pretreatment methods for the sugars to be hydrolyzed due to the structural and protective role of cellulose and lignin in plants.

Composition of lignocellulosic biomass

Lignocellulosic biomass consists of an intermeshed and chemically bonded complex of three main polymers, namely cellulose, hemicelluloses and lignin (Hendriks & Zeeman, 2009; Howard *et al.*, 2003) (Figure 1). Cellulose is the major constituent of lignocelluloses. It is a linear polymer composed of thousands of D-glucose subunits linked by β-(1–4)-glycosidic bonds. In plants, the cellulose structure consists of a crystalline (organized) structure and a not well-organized, amorphous structure which are bundled together to form cellulose fibrils or cellulose bundles (Hendriks & Zeeman, 2009). Each independent cellulose bundle is weakly bound together through hydrogen bonding. The structural conformation of cellulose, as well as its close association with lignin, hemicellulose, starch, protein and minerals renders cellulose highly resistant to hydrolysis (Aristidou & Penttilä, 2000; van Maris *et al.*, 2006).

Hemicellulose, the second major constituent of lignocelluloses, is a highly branched and complex heteropolymer that contains hexoses (D-glucose, D-galactose, D-mannose, L-rhamnose, L-fucose), pentoses (D-xylose and L-arabinose) and uronic acids (D-glucuronic acid and D-galacturonic acid). The hemicellulose composition is strongly dependent on the plant source, with xylan as the dominant component in hardwoods, whereas glucomannan is the major hemicellulose component in softwoods (Hendriks & Zeeman, 2009). However, in contrast to cellulose, hemicellulose branches into short lateral chains that consist of different sugars that are easily hydrolyzed to their constituent

monosaccharides (Hendriks & Zeeman, 2009; Aristidou & Penttila, 2000). Hemicellulose serves as a connection between the lignin and the cellulose fibres and gives the whole cellulose-hemicellulose-lignin network more rigidity.

Lignin is one of the most abundant polymers in nature and is present in the cellular wall of plants, giving the plant structural support and resistance to microbial attack. Lignin is an aromatic polymer containing three different phenylpropane units such as trans-ρ-coumaryl alcohol, trans-ρ-coniferyl alcohol and trans-ρ-sinapyl alcohol (Hahn-Hägerdal *et al.*, 1991; Hendriks & Zeeman, 2009; van Maris *et al.*, 2006). While the lignin fraction does not contribute fermentable carbon sources, it is relevant as a potential source of microbial inhibitors.

Generally, in usual lignocellulosic biomass, pectin is less prominent than cellulose and hemicelluloses. However, some agricultural wastes such as citrus peels and sugar beet pulp are extremely rich in pectin (van Maris *et al.*, 2006). Pectins are complex and heterogeneous polymers that primarily act as hydrating and cementing agents for the cellulosic matrix of plant cell walls. The principal unit in pectin chains is α-(1-4) linked galacturonic acid. The galacturonic acid residues can be esterified with methyl and acetyl groups. Furthermore, pectin contains the branched polysaccharides rhamnogalacturonan I, rhamnogalacturonan II and xylogalacturonan (Blanco *et al.*, 1999; van Maris *et al.*, 2006).

Bioconversion of lignocellulosic biomass

Lignocellulose constitutes a major part of plant biomass. This vast resource has diverse biotechnological potential in the production of value added products such as biofuels, biofertilizers, animal feed products, biochemicals, biopesticides, biopromoters and biotransformation of the biomass itself into compost or biopulp (Tengerdy & Szakacs, 2003). To fully utilize the potential of lignocellulosic biomass, hydrolysis of lignocellulose into fermentable sugars by physical, chemical, physicochemical and biological pretreatment methods is the primary requirement in all applications. During pretreatment, lignin is removed and the porosity of the lignocellulosic materials increased to release the cellulose and hemicellulose sugars. Inhibitors are also formed in the hydrolysates.

Complete substrate utilisation by the micro-organism and inhibitor tolerance are the prerequisites to render lignocellulosic microbial bioconversion processes economically competititve (Hahn-Hägerdal *et al.*, 2007). However, owing to the chemical and structural complexity of lignocellulosic biomass, the sustainable utilization of lignocelluloses is limited until it undergoes pretreatment. This usually followed by enzymatic hydrolysis, during which oligomeric sugars such as cellulose are broken down to monomeric sugars.

Pretreatment and hydrolysis of lignocellulosic biomass

The goal of the pretreatment process is to alter the recalcitrant structure of lignocellulosic biomass to increase the availability of degradeable carbohydrates present in biomass. A pretreatment method is regarded as an effective method based on a number of features such as a high recovery of all carbohydrates, the production of limited amounts of by-products that are inhibitory to the subsequent hydrolysis and bioconversion processes, minimum energy consumption and cost effectiveness (Kumar *et al.*, 2009).

Physical pretreatment methods

Physical pretreatment methods refer to mechanical and non-mechnical methods. A size reduction step is necessary before most chemical and biological pretreatment processes, while it increases the surface area of the cellulose and thus improves the enzymatic hydrolysis rate. Mechanical pretreatment involves chipping, grinding and milling to break down the lignocellulosic biomass into finer particles (Galbe & Zacchi, 2007; Kumar *et al*., 2009). Non-mechanical pretreatment refers to methods such as the use of gamma rays to render the biomass more accessible to hydrolysis, but this not a cost effective method (Kumar *et al.*, 2009).

Chemical pretreatment methods

Chemical pretreatment methods include the use of acids, alkalis, ozone or hydrogen peroxide, to list a few, to break down the polysaccharides found in lignocellulosic biomass.

Dilute acid pre-treatment

Dilute sulphuric acid is the most commonly used approach since it is cheap and effective (Galbe & Zacchi, 2007). Nitric acid, hydrochloric acid and phosphoric acids have been tested as well. Dilute acid pretreatment acts by solubilising the hemicellulose components into monomeric sugars and rendering the cellulose more accessible to enzymatic hydrolysis (Kumar *et al.*, 2009; Mosier *et al.*, 2005). Acid pretreatments are performed at a low pH and this has an effect on the severity of the method, with more severe conditions during pretreatment leading to a greater degradation of hemicellulosic sugars, a lower recovery of sugars and an enhanced formation of furfurals, which are not desirable (Galbe and Zacchi, 2007).

Alkaline pretreatment

This form of pretreatment is based on soaking the biomass in alkaline solutions such as NaOH, KOH, NH_4OH, or $Ca(OH)_2$ for a certain period of time at required temperatures (Taherzadeh & Karimi, 2008). This leads to swelling of plant pores that result in an increase of the internal surface area and decrease of crystallinity of cellulose (Galbe & Zacchi, 2007). The mechanism of alkaline pretreatment is understood to be saponification of intermolecular ester bonds cross-linking xylan hemicelluloses and other components such as lignin (Sun & Cheng, 2002). Sodium hydroxide is the most commonly studied pretreatment alkali and is seen as an alternative to sulphuric acid (Kumar *et al.*, 2009; Silverstein *et al.*, 2007). However, it is much slower and the pretreatment times are in the order of hours and sometimes days rather than minutes and seconds (Kumar *et al.*, 2009). Ammonia is used in the AFEX pretreatment process. An advantage of alkali pretreatment is that it removes acetyl and various uronic acid substitutions formed during the degradation of hemicelluloses, which usually lower the accessibility of the hemicelluloses and cellulose surface to the enzyme during enzymatic hydrolysis (Mosier *et al.*, 2005).

Ozonolysis

The objective of this method is to degrade lignin, thus increasing the digestibility of the lignocellulosic biomass. The degradation is essentially limited to lignin. However, it was found that hemicellulose was slightly degraded, but cellulose was hardly affected (Sun & Cheng, 2002). The advantages of ozonolysis pretreatment are effective lignin removal, no formation of toxic residues for the downstream processes and the reactions are carried out at room temperature and

pressure. However, a large amount of ozone is required, making the process expensive (Kumar et al., 2009; Sun & Cheng, 2002).

Oxidative delignification

The oxidative delignification process involves the addition of an oxidizing compound such as H_2O_2 or peracetic acid to the biomass for lignin biodegradation (Hendriks & Zeeman, 2009). Hydrogen peroxide pretreatment acts by solubilizing the lignin through an oxidative process, thus loosening the lignocellulosic structure and leads to improved enzymatic conversion. However, the decomposition of hydrogen peroxide in the presence of water at high temperatures has been reported, which results in decreased lignin and xylan solubilization (Silverstein *et al.*, 2007).

Organosolv process

The organosolv (organosolvation) process employs an organic or aqueous organic solvent mixture with inorganic catalysts such as HCl or H_2SO_4 to break the internal lignin and hemicellulose bonds (Sun & Cheng, 2002; Zhao *et al.*, 2009). Methanol, ethanol, acetone, ethylene glycol and tetrahydrofurfuryl alcohol (THFA) are common organic solvents that can be used in the process. Cellulose is partially hydrolysed into smaller fragments that remain insoluble in the liquor, whereas hemicellulose is hydrolysed mostly into soluble components such as oligosaccharides, monosaccharides and acetic acid, while lignin is hydrolysed primarily into lower molecular weight fragments that dissolve in the aqueous ethanol liquor (Duff & Murray, 1996; Kumar *et al.*, 2009).

After pretreatment, the solvents used need to be drained from the reactor, evaporated, condensed and recycled to reduce operating costs. Moreover, removal of solvents from the system is necessary to prevent them inhibiting enzymatic hydrolysis, growth of microorganisms as well as fermentation (Sun & Cheng, 2002).

Physicochemical pretreatment methods

Physicochemical pretreatment methods include methods that are a mixture of physical and chemical pretreatment methods. The most commonly used physicochemical methods for the pretreatment of lignocellulosic biomass are steam explosion and ammonia fiber explosion (AFEX) methods (Kumar *et al*., 2009)

Steam pretreatment

Lignocellulosic biomass is heated with high pressure saturated steam and then the pressure is quickly decreased. This forces the biomass to undergo explosive decompression. The biomass is usually subjected to high temperature (160–260ºC) and high pressure saturated steam (0.69–4.83 MPa) for several seconds or a few minutes before the material is exposed to atmospheric pressure (Sun & Cheng, 2002). The role of the steam is to disrupt the hemicellulose, thus improving the accessibility of enzymes to the cellulose fibrils during hydrolysis (Mosier *et al*. 2005).

The addition of H_2SO_4, SO_2 or CO_2 in steam explosion can effectively improve enzymatic hydrolysis, decrease the production of inhibitory compounds, and lead to more complete removal of hemicelluloses. The disadvantage of using steam pretreatment is its high cost due to expensive equipment required (Sun & Cheng, 2002).

Ammonia fibre explosion pretreatment (AFEX)

Ammonia fibre explosion pretreatment operates on a similar concept to steam pretreatment, whereas in this instance lignocellulosic biomass is exposed to liquid ammonia at high temperature and pressure for a period of time, and then the pressure is quickly released (Galbe & Zacchi, 2007; Sun & Cheng, 2002). The biomass is subjected to liquid ammonia (1-2 kg ammonia per kg of dry biomass) at temperatures below 100ºC and a pressure above 3 MPa for 10-60 min. During pretreatment, only a small amount of the hemicelluloses is solubilised and lignin is not removed. However, the pretreatment alters the structure of the biomass, resulting in an increased water retention capacity and improved digestibility (Galbe & Zacchi, 2007). The AFEX pretreatment method has been used for herbaceous and agricultural residues including alfalfa, wheat straw, corn stover, municipal solid waste, switchgrass and sugarcane bagasse. However, this method only works moderately well on hardwoods and is not effective on materials with a higher lignin content such as newspaper and aspen chips which contain up to 25% lignin (Mosier *et al.*, 2005; Sun & Cheng, 2002).

Biological pretreatment

Biological pretreatment processes exploit the ability of microorganisms such as white-rot, brown-rot and soft-rot fungi to degrade lignin (Sun & Cheng, 2002). White and soft rots attack both cellulose and lignin, whereas brown rots attack mainly cellulose. White-rot fungi are the most effective basidiomycetes for the biological pretreatment of lignocellulosic biomass. Lignin degradation by white-rot fungi, specifically *Phanerochaete chrysosporium*, *Pleurotus ostraetus* and *Trametes versicolor*, is an oxidative process that is catalyzed by lignin peroxidases, manganese peroxidases and laccases, which are regarded as key enzymes for this process (Lee *et al.*, 2007). Biological pretreatment is considered to be environmentally friendly and energy saving since it requires no chemicals and is performed at low temperatures. However, the rate of hydrolysis is very slow and some material is lost as these organisms to an extent can consume cellulose and hemicelluloses (Galbe & Zacchi, 2007).

Enzymatic hydrolysis of cellulose

The subsequent step after pretreatment is enzymatic hydrolysis whereby cellulose and other structural carbohydrates are split into their component sugars that can be utilised by microorganisms. Enzymatic hydrolysis of cellulose is carried out by cellulases which are highly specific and the products of the hydrolysis are usually reducing sugars including glucose (Sun & Cheng, 2002). The enzymatic hydrolysis is usually conducted at mild conditions (pH 4.8 and temperature 45–50 ^{0}C) and does not result in a corrosion problem (Duff & Murray, 1996). Bacteria and fungi can synthesize cellulases under aerobic or anaerobic conditions. The major source of the commercial cellulases is from the aerobic cultivation of the filamentous fungus Trichoderma reesei, which produces the most effective enzymes for the hydrolysis of crystalline cellulose (Sun & Cheng, 2002).

The term cellulases refers to enzymes from three major groups: (i) endoglucanase (EG, endo-1,4-D-glucanohydrolase), which randomly attacks the regions of low crystallinity in the cellulose chain, creating free chain ends; (ii) exoglucanase or cellobiohydrolase (CBH, 1,4-β-D-glucan

cellobiodehydrolase), which degrades the cellulose further by removing cellobiose units from the free chain ends; (iii) β-glucosidase, which hydrolyses cellobiose to produce two glucose molecules (Duff & Murray, 1996; Lynd *et al.*, 2002; Prasad *et al.*, 2007).

Products from lignocellulosic biomass

Bioethanol

Bioethanol production is considered one of the most predominant uses of lignocellulosic biomass. The production of bioethanol from lignocellulosic feedstocks is essential to replace starchy feedstocks, which are primary food sources for humans and feed for animals. After pretreatment and enzymatic hydrolysis, fermentation of the sugars released can be carried out by *Saccharomyces cerevisiae,* which ferments glucose to ethanol but does not utilise pentoses. *Pichia stipitis* can utilize and ferment D-xylose and genes from it have been cloned into *S. cerevisiae* (Hahn-Hägerdal *et al.*, 2007; Jeffries & Jin, 2004). *Escherichia coli* KO11, genetically modified with genes from *Zymomonas mobilis,* has been employed as an ethanologenic strain to ferment both glucose and xylose (Dien *et al.*, 1997; Dien *et al.*, 2003). *Zymomonas mobilis* has also been genetically modified for utilising various sugars for ethanol production. A microorganism capable of utilising all sugars present in the feedstock is needed for improving the ethanol yield and thus the economics of the bioethanol process (Hahn-Hägerdal *et al.*, 2007).

Enzyme production

Lignocellulolytic enzyme complexes containing cellulases, hemicellulases, pectinases and ligninases have numerous industrial applications. Currently filamentous fungi are used for the production of cellulases and xylanases (Duff & Murray, 1996; Haltrich *et al.*, 1996; Tengerdy & Szakacs, 2003). Xylanases are used commercially in the pulp and paper, food, and animal feed industries. In the pulp and paper industry, xylanases enhance the bleaching of pulp, decreasing the concentration of chlorine-containing compounds in the process and the subsequent discharge of organochlorines in the effluent (Beg *et al.*, 2000; Viikari *et al.*, 1994). In the food industry, xylanases are used to enhance the baking of cookies, cakes, crackers, and other foods by improving the breakdown of polysaccharides in the dough. In animal feeds, xylanase aids the digestibility of wheat by poultry and swine by decreasing the viscosity of the feed. Most commercial xylanases are produced by *Trichoderma, Bacillus, Aspergillus, Penicillium, Aureobasidium, Humicola* and *Talaromyces* species. Additionally, lignocellulolytic fungi may also produce other enzymes such as proteases, lipases and phytases on lignocellulosic substrates, in single or mixed cultures (Tengerdy & Szakacs, 2003).

Animal feed production

Microbial bioconversion of lignocellulosic biomass into single cell protein (SCP) or by microbial protein enrichment of biomass for animal feed has been practiced for ages. This has been carried out using a number of methods such as ensilaging, submerged fermentation and solid state fermentation (Azuolay *et al.*, 1980; Nigam, 1998). The predominant method is either by submerged fermentation (SF) for single cell protein production or solid state fermentation (SSF). There are reports that large-scale enrichment of lignocellulose to microbial protein by SSF proved to be non-economical, as has been the case with single cell protein

production by traditional SF (Tengerdy & Szakacs, 2003).

Opuntia ficus-indica biomass as carbon feedstock

Crops with the ability to conserve water offer a distinct agricultural advantage in arid and semi-arid regions. One of such crops is the prickly pear cactus (*Opuntia ficus-indica*). The taxonomic genus *Opuntia*, which belongs to the subfamily Opuntioideae, family cactaceae, is a xerophyte consisting of about 200 to 300 species (Stintzing & Carle, 2005). *Opuntia* species have adapted perfectly to arid (less than 250 mm annual precipitation) and semi-arid zones (250–450 mm annual precipitation) characterized by drought conditions, erratic rainfall and poor soils subject to erosion, having developed phenological, physiological and structural adaptations to sustain their development in these adverse environments (Nefzaoui & Ben Salem, 2002; Stintzing & Carle, 2005). The *O. ficus-indica* cactus originates from the American continent and it is cultivated mainly for fruit production (Nobel *et al.*, 1992). Young shoots are also eaten as a vegetable (nopalitos) in Mexico and southern USA, whereas in North Africa the cultivation of *O. ficus-indica* is used against soil erosion in arid areas as well as a forage substitute during drought (Malainine *et al.*, 2003). *Opuntia* cultivars have notable adaptations for arid and semi-arid environments, namely their asynchronous reproduction and their crassulacean acid metabolism (CAM) that enables them to grow at a very high efficiency under conditions of limited water. Combined with structural adaptations such as succulence, this allows the plant to continue assimilation of carbon dioxide and reach acceptable productivity levels even in years of severe drought (Nefzaoui & Salem, 2002). Hence, *Opuntia* species contributes in times of drought as life saving crops for both humans and animals. Some species are even naturalized weeds in South Africa and Australia, where the environmental conditions are particularly favourable (Brutsch & Zimmerman, 1993). *Opuntia* species have many potential uses: the fruits are of economic value whereas the cladodes (stems) are consumed by humans as napolitas, used as animal feed and also have medicinal uses, to mention a few (Hamdi, 1997; Hamdi, 2006; Stintzing & Carle, 2005).

In recent years, plantations for fruit or forage production, as well as for use as vegetable (nopalitos) and cochineal dye production, have been developed in many countries of Africa, America, Asia and Europe. There is increasing interest in *Opuntia* and *O. ficus-indica* in particular. *Opuntia* species have become a constant source of products and functions, originally as a wild plant and presently as a crop for both subsistence and market-oriented agriculture, contributing to the food security of populations in agriculturally marginalized areas. It was only from 1920-1930 onwards that cultivation of *Opuntia* spp. for fodder production was established, mainly based on *Opuntia ficus-indica* f. *inermis* (spineless cactus) (Nefzaoui & Ben Salem, 2002). *Opuntia* is a source of highly digestible energy, water and minerals for animals, but needs to be combined with a better protein source to constitute a complete animal feed (de Kock, 2002; Nobel, 2002). For farmers in arid zones, *Opuntia* planting is one solution to alleviate the problem of recurrent droughts. The succulence and nutritive value of *Opuntia* make it a valuable emergency crop, permitting livestock farmers in Brazil, Mexico, South Africa and USA to survive prolonged and severe droughts (de Kock, 2002). Prickly pear

cactus cladodes are used as fodder for ruminant animals, but require supplementation with other protein rich forage because of the low protein content of the cladodes. This renders the prickly pear cladodes not suitable for use as sole protein livestock feed (de Kock, 2002). The protein content of the cladodes could be improved by single cell protein production; thus the cladodes have potential of serving as lignocellulosic feedstock for microbial cultivation.

Opuntia ficus-indica in South Africa

The exact date when *Opuntia ficus-indica* (L.) Miller was introduced to southern Africa is not clear, although it was possibly during the early European settlement of the Cape in the 17th century (Annecke & Moran, 1978; Brutsch & Zimmermann, 1993). In South Africa, the prickly pear industry includes both wild spiny prickly pear and cultivated spineless prickly pear. During recent years the spiny prickly pear has been declared a naturalized weed and its eradication has been enacted by law. However, the spineless form was preserved (Brutsch & Zimmermann, 1993). In the past, there had been heavy infestations of spiny prickly pear on about 900 000 ha of veld, with the heaviest infestations in the Eastern Cape and Karoo. However, with the introduction since 1932 of insect enemies such as *Dactylopius coccus* for the biological control of this plant, dense infestations have largely been eliminated (Brutsch & Zimmerman, 1993). The commercial exploitation of the spineless cactus had been achieved in a wide range of climates. However, there is an influence of climate on the distribution of the wild spiny cactus pear (Brutsch, 1997). The exploitation of fruit from wild *Opuntia ficus-indica* in South Africa has been ongoing for hundreds of years. Much of the developing cactus-pear industry in South Africa is based on spineless cultivars of *Opuntia ficus-indica* introduced into the country during 1914 (Brutsch & Zimmermann, 1993). Some of these included the various Burbank spineless varieties, primarily utilized for fodder. Currently, it is estimated that South Africa has approximately 1 500 hectares of prickly pear under intensive cultivation, which yield about 15 000 tonnes of prickly pear per annum (Swart & Swart, 2003).

Composition of *Opuntia ficus-indica* fruits and cladodes

The composition of *O. ficus-indica* fruits and cladodes depends especially on the horticultural practices and the level of maturation. The composition of cladodes may vary from different geographical zones, depending on the soil properties at the cultivation site, the season and age of the plant (Retamal *et al.*, 1987; Rodriguez-Felix & Cantwell, 1988; Stintzing *et al.*, 2005).

Composition of fruits

The fruit is a pear shaped multiseeded berry with a thick pericarp having a number of clefts of small prickles enclosing a red, violet, green or orange yellow, sweet, luscious pulp, intermixed with a number of small, black, shiny seeds. *Opuntia* fruit weighs approximately 30-70 g and the overall composition of the pear fruit is 48% peel, 45% strained pulp and 7% seeds (Hamdi, 2006; Merin *et al.*, 1987). The pH of the pulp is a slightly acidic 5.75 and the total solids content is 16%, which is comparable to common fruit pulps such as apricots, apples, cherries, and plums (Hamdi, 1997; Sawaya *et al.*, 1983). The composition of the soluble carbohydrates has glucose and fructose as the predominant sugars in the pulp at a 60:40 ratio. They

constitute 10 to 13% of the fresh weight and more than 50% of the pulp dry weight (El Kossori *et al.,* 1998).

Uses of prickly pear fruits

Human and animal food: In semi-arid zones of Mexico, prickly pear has become an important fruit crop mostly for subsistence farmers (Pimienta-Barrios, 1994). Fresh fruits serve as a source of carbohydrates and vitamins in human nutrition. In southern California, the Indians dry large quantities of prickly pear fruit and use them as relish. The fresh pulpy pears have good dietetic properties because they aid digestion and provide dietary fibres, organic acids and certain vitamins (Hamdi, 1997). Over 50% of the fruit (peel and seeds) are by-products and used as animal feed.

Manufacture of jam: Both prickly pear juice and preserves are canned in the manufacturing of prickly pear jam (Sawaya *et al.,* 1983). The jam has a desirable taste with or without addition of any flavour compounds. Traditionally, the prickly pear juice has been used to prepare such commercial foods as tuna honey, melcocha pastry and tuna queso cake (Hamdi, 1997).

Source of natural food pigments:

The prickly pear juice contains betanine, isobetanine and betalainic glucoside which are responsible for the red beet colour of the fruit (Hamdi, 1997).

Production of single cell protein (SCP):

Prickly pear juice contains fermentable sugars (glucose and fructose) and serves as a suitable substrate for SCP production. The production of SCP using *Candida utilis* cultivated on prickly pear juice as the carbon source in batch and continuous cultivation has been studied (Paredes-Lopez *et al.,* 1976). In batch culture, the maximum specific growth rate (μ_{max}) and the biomass yield coefficient ($Y_{x/s}$) of *C. utilis* varied according to sugar concentration. At 1% sugar, μ_{max}, the biomass concentration and $Y_{x/s}$ values were 0.47 h^{-1}, 4.84 g l^{-1} and 0.426 respectively. The best yields occurred in the chemostat culture at pH 3.5 to 4.5 at 30°C during cultivation at a dilution rate of 0.55 h^{-1}.

Production of alcoholic drinks: Traditional alcoholic drinks are produced from prickly pear juice in Mexico such as colonche and pulque (Hamdi, 1997). The final ethanol concentration obtained from diluted prickly pear juice was 5.45% v/v.

Composition of cladodes

The major components of cladodes are carbohydrate-containing polymers, which consist of a mixture of mucilage and pectin. The cladodes, just as other vegetables, have a low protein and fat content but the crude fibre content is higher than that of most other vegetables (Hamdi, 2006; Inglese *et al.,* 2002). The composition of cladodes varies depending on the soil properties at the cultivation site, the season and age of the plant (Retamal *et al.,* 1987; Rodriguez-Felix & Cantwell, 1988; Stintzing *et al.,* 2005). Generally, fresh cladodes have a high moisture content of up to 92% due to their crassulacean acid metabolism and stem tissues containing numerous mucilaginous cells that store large volumes of water. Other constituents are carbohydrates (5.1%), lipids (0.2%), minerals (1.9%), and protein (0.8%) (Hamdi, 1997). There is a considerable variation in the literature regarding the chemical composition of *O. ficus-indica* cladodes. Therefore, data on the respective constituents should not be taken as absolute values (Nefzaoui & Ben

Salem, 2002; Stintzing & Carle, 2005). The composition of de-barbed cladodes, as reported by Malainine *et al.* (2005), are 19.6 g ash, 7.2 g fats and waxes, 3.6 g lignin, 21.6 g cellulose, and 48 g other polysaccharides per 100 g (dry wt), but crude protein was not determined. Table 5 shows the typical range of constituents of *O. ficus indica* cladodes.

On a dry biomass basis, *O. ficus-indica* cladodes contain approximately 22% cellulose, 13% hemicellulose and 34% acidic polysaccharides (mucilage and pectins), giving roughly 69% total carbohydrates (Malainine *et al*., 2005).

Mucilage component

The cactaceae family is characterized by the production of a hydrocolloid commonly referred to as mucilage (Matsuhiro *et al.*, 2006), which forms molecular networks that have the ability of retaining large amounts of water (Sepulveda *et al*., 2007). Mucilage is present in both the cladode and fruit of the plant. In the cladode it is stored in mucilaginous cells that are located within the chlorenchyma and parenchyma (Saenz, 2004). The chemical composition of mucilage in *O. ficus-indica* cladodes has been the subject of various studies by several authors. In general, the mucilage is a high molecular weight polysaccharide comprising variable amounts of L-arabinose, D-galactose, L-rhamnose and D-xylose as the major neutral sugars, as well as D-galacturonic acid (Medina-Torres *et al.*, 2000; Sáenz *et al.*, 2004; Sepúlveda *et al.*, 2007). According to Nobel *et al*. (1992), the sugar composition in *O. ficus-indica* mucilage was approximately (dry weight) 42% arabinose, 22% xylose, 21% galactose, 8% galacturonic acid and 7% rhamnose. Similar results were also reported by Medina-Torres *et al*. (2000).

The suggested primary structure describes the mucilage as a linear repeating core chain of (1→4) linked α-D-galacturonic acid and (1→2) linked β-L-rhamnose with lateral chains of (1→6)-β-D-galactose attached to O-4 of rhamnose residues. The galactose side residues present further branching in the O-3 or both the O-3 and O-4 positions (McGarvie & Parolis, 1979a). The composition of the acid-labile peripheral chains is complex with at least 20 different types of oligosaccharides, mostly disaccharides and trisaccharides being identified. These invariably contain (1→5) linked L-arabinose residues and D-xylose as terminal groups, giving a xylose:arabinose ratio of approximately 1:2. Rhamnose and galacturonic acid are presumed to be confined to the acid resistant backbone of the mucilage as they do not appear after the mucilage hydrolysis (McGarvie & Parolis, 1979b).

Economic uses of prickly pear cladodes

Opuntia cladodes are potential sources of foods and functional components such as dietary fibre, natural colourants and antioxidants.

Human food: The young cladodes, called "nopalitos", are consumed as vegetables in Mexico and in the southwestern United States (Sáenz, 2002). The prickly pear pads are harvested, cleaned, cut into small pieces and it is either sold fresh or preserved by various methods. Further products derived from cladodes are jam, chutney, candied nopales, etc. (Saenz, 2002; Stintzing & Carle, 2005). Based on the determination of ten amino acids, the biological value of prickly pear cactus pads was 72.6% relative to egg protein (Hamdi, 1997).

Alcohol production from cladodes

Ethanol production by *Saccharomyces cerevisiae* using prickly pear cladodes as a substrate was carried out after enzymatic hydrolysis using cellulase followed by acid hydrolysis of substrate (Retamal *et al.*, 1987). The highest ethanol concentration obtained by direct fermentation was 1.4% (w/v).

Cochineal production: Cochineal is produced by drying and milling adult female *Dactylococcus coccus* Costa, a parasitic insect of the prickly pear cladodes. One kilogram of cochineal could be obtained from 140 000 insects, amounting to a pigment content of 50% of their total weight (Stintzing *et al.*, 2005). Cochineal is a pigment blend mainly consisting of carminic acid (hydroxyanthraquinone derivative), but also kermesic and flavokermesic acids. Carminic acid is useful in the food industry for its natural red colour and also finds application in the cosmetic and textile industry. The worldwide production of cochineal has increased with Peru, the Canary Islands and Chile as the main producers, followed by Mexico, Bolivia, South Africa and Argentina (Stintzing *et al.*, 2005). However, cochineal is presently replaced by cheaper synthetic alternatives or by other natural pigments of plant origin.

Animal fodder: Prickly pear cactus is useful not only because it can withstand drought, but also because its conversion efficiency is greater than C_3 grasses and C_4 broad leaf plants. Biomass generation per unit of water is on average about three times higher than for C_4 plants and five times higher than for C_3 plants (Nefzaoui & Ben Salem, 2002). Under optimal conditions, the various types of plants can produce similar amounts of dry matter per surface area, but under arid and semi-arid conditions, CAM plants are superior to C_3 and C_4 plants. The cladodes of cacti, and specifically *O. ficus-indica*, have been exceptionally useful livestock forage in times of drought, primarily by providing digestible energy, water and vitamins. Although mainly used for cattle, *O. ficus-indica* has also been used as forage for pigs. However, the cladodes must be combined with other forages to complete the daily diet because *Opuntia* biomass is poor in protein, although rich in carbohydrates and calcium (de Kock, 2002). *Opuntia* biomass also has a laxative effect on livestock when consumed in large amounts, especially when it is the major feed. This is attributed to a high oxalic acid content in the cladodes (de Kock, 2002). Since it grows in severely degraded land, its use is important because of its abundance in areas where few crops can grow. It is estimated that, worldwide, 900 000 ha are under cultivation with *Opuntia* species for forage production (Ben Salem *et al.*, 2002). Whereas spineless types need to be protected against animal grazing, the more cold-hardy, slower growing spiny types require no such protection, although it is necessary to burn off the spines before using for livestock feed.

The Tunisian government showed that feeding livestock with *O. ficus-indca* cladodes during drought was two to three-fold times cheaper than conventional forage. However, the addition of straw or alfalfa hay to the cladodes before feeding livestock is recommended (de Kock, 2002). In South Africa, an ensilage of 84% minced cladodes plus 16% lucerne and straw hay gave a product of high quality that maintained a good level of milk production by cows (Hamdi, 1997). However, the protein content of the cladodes could be improved by single cell protein production. The efficient utilization of this abundantly

available biomass to produce low cost SCP will reduce the dependency on commercial protein concentrates for livestock feeds and thus improve food security. Single cell protein from lignocellulosic agricultural biomass can serve as a superior source of high quality supplemental protein in livestock feeds (Rajoka, 2005).

Results and Discussion

In spite of the abundant supply and uses of the prickly pear cladodes in South Africa, it is of limited use as animal fodder due to its low protein content. Moreover, it is not a desirable stand-alone animal feed because of its high oxalic content and the laxative action this compound causes in cattle. The use of cladodes is limited to drought periods and with the increasing awareness of the effect of climate change and desertification, this may serve as a useful animal fodder if the protein content can be improved. Protein enrichment of prickly pear cladodes through yeast single cell protein production has the potential of yielding a well-balanced animal feed. This will improve the protein quality of animal feed and reduce the cost of procurement of protein concentrates in animal feed formulations.

There are very few reports on the single cell protein production from prickly pear to provide a protein enriched animal fodder. Most of the protein enrichment of cladodes on farms were carried out by the less cost effective traditional ensilaging.

Table.1.1 Average composition of microorganisms (% dry weight).
Adapted from Miller & Litsky (1976)

Constituent	Filamentous fungi	Algae	Yeasts	Bacteria
Protein	30-45	40-60	45-55	50-65
Fat	2-8	7-20	2-6	1.5-3.0
Ash	9-14	8-10	5-9.5	3-7
Nucleic acids	7-10	3-8	6-12	8-12

Table.1.2 Essential amino acid content of microorganisms of interest for SCP production (g per 16 g N) compared to conventional protein sources
(Lichtfield, 1979; Boze *et al.*, 1992)

Protein Source	Carbon substrate	Cys	Ile	Leu	Lys	Met	Phe	Thr	Try	Val
FAO reference		2.0	4.2	4.8	4.2	2.2	2.8	2.8	1.0	4.2
Soybean meal		0.7	2.2	3.5	2.8	0.6	2.2	1.9	0.6	2.3
Fishmeal		0.7	3.2	5.0	4.9	1.9	2.9	3.0	0.9	3.7
Egg		2.4	6.7	8.9	6.5	5.1	5.8	5.1	1.6	7.3
Algae										
Chlorella sorokiniana	CO_2	_	3.4	4.0	7.8	1.8	2.7	3.2	1.4	5.1
Spirulina maxima	CO_2	0.4	5.8	7.8	4.8	1.5	4.6	4.6	1.3	6.3

Bacteria and Actinomycetes										
Cellulomonas alcaligenes	Bagasse	_	5.4	7.4	7.6	2.0	4.7	5.5		7.1
Methylophilus methylotrophus	Methanol	0.6	4.3	6.8	5.9	2.4	3.4	4.6	0.9	5.2
Thermomonospora Fusca	Cellulose pulp	0.4	3.2	6.1	3.6	2.0	2.6	4.0	_	13.0
Yeasts										
Candida lipolytica	*n*-Alkanes	1.1	4.5	7.0	7.0	1.8	4.4	4.9	1.4	5.4
Candida utilis	Ethanol	0.4	4.5	7.1	6.6	1.4	4.1	5.5	1.2	5.7
Kluyveromyces fragilis	Whey	_	4.0	6.1	6.9	1.9	2.8	5.8	1.4	5.4
Saccharomyces Cerevisiae	Molasses	1.6	5.5	7.9	8.2	2.5	4.5	4.8	1.2	5.5
Filamentous fungi										
Aspergillus niger	Molasses	1.1	4.2	5.7	5.9	2.6	3.8	5.0	2.1	5.2
Morchella crassipes	Glucose	0.4	2.9	5.6	3.5	1.0	1.9	3.0	1.5	3.0
Paecilomyces variotii	Sulphite waste liquor	1.1	4.3	6.9	6.4	1.5	3.7	4.6	1.2	5.1

Table.1.3 Microorganisms used for SCP production, listed according to carbon source (Boze *et al.*, 1992).

Substrate	Microorganism
	Algae
CO_2	*Chlorella pyrenoidosa, C. regularis, C. sorokiniana, Oocystis polymorpha, Scenedesmus quadricaula, Spirulina maxima, S. plantensis, Dunaliella bardawil*
	Bacteria, including actinomycetes
n- Alkanes	*Acinetobacter cerificans, Achromobacter delvacuate, Mycobacterium phlei, Nocardia* sp., *Pseudomonas* sp.
Methane	*Corynebacterium hydrocarbonoclastus, Nocardia paraffinica, Acinetobacter* sp., *Flavobacterium* sp., *Hyphomicrobium* sp., *Methylomonas methanica, Methylococcus capsulatus*
Methanol	*Methylomonas methylovora, M. clara, M. methanolica, Flavobacterium* sp., *Methylophilus methylotrophus, Pseudomonas* sp., *Streptomyces* sp., *Xanthomonas* sp.
Ethanol	*Acinetobacter calcoaceticus*
Cellulosic wastes	*Thermonospora fusca*
Sulphite waste liquor	*Pseudomonas denitrificans*
	Yeasts
n-Alkanes	*Candida lipolytica, C. tropicalis, C. guilliermondii, C. maltosa, C. paraffinica, C. oleophila, Yarrowia lipolytica*
Methanol	*Candida utilis, Hanseniaspora sp., Pichia pastoris, Hansenula sp., Kloeckera sp.*

Table 1.3 (continued)

Substrate	**Microorganism**
Whey	*Kluyveromyces fragilis, Candida intermedia*
Cane molasses	*Saccharomyces cerevisiae*
Starch	*Schwanniomyces alluvius, Lipomyces kononenkoeae*
Lipids	*Candida rugosa, C. utilis, C. lipolytica, C. blankii, C. curvata, C. deformans, C. parapsilosis*
Sulphite waste liquor	*Candida utilis, C. tropicalis*
	Filamentous fungi
Glucose	*Agaricus blazei, A. Campestris*
Malt-molasses	*Agaricus campestris*
Starch	*Aspergillus niger, Fusarium venenatum "graminearum"*
Sulphife waste liquor	*Paecilomyces variotii*
Cellulose	*Chaetomium cellulolyticum, Trichoderma viride*
Brewery waste	*Calvatica gigantean*
Carob bean extract	*Aspergillus niger, Fusarium moniliforme*

Figure.1.1 General structure of cellulose, hemicellulose and lignin biopolymers found in lignocellulosic biomass (Chang, 2007).

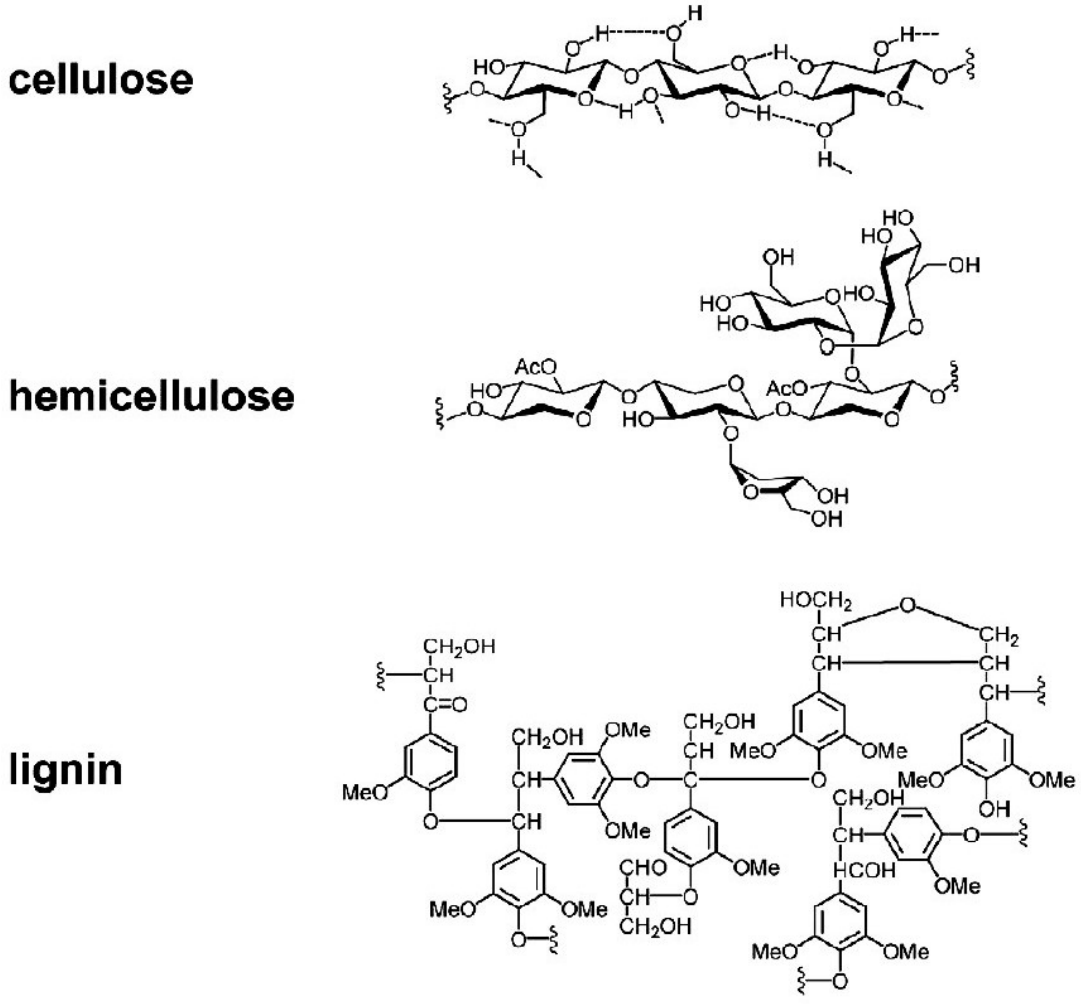

Table.1.4 Crude composition of the prickly pear fruit (% w/w, dry matter) (El Kossori *et al*., 1998)

Constituent	Pulp	Skin
Protein	5-13	8.3
Non-protein nitrogen	0.025	0.024
Lipids	0.97	2.43
Total fibre	20.5	40.8
Ash	8.5	12.1
Ethanol soluble carbohydrates	58.3	27.6
Starch	4.55	7.12

Table.1.5 Mean chemical composition of despined *Opuntia ficus-indica* cladodes (adapted from Stintzing & Carle, 2005)

Constituent	Fresh weight (g/ 100g)	Dry weight (g/ 100g)
Water	88-95	-
Carbohydrates (total polysaccharides)	3-7	64-71
Ash	1-2	19-23
Crude fibre	1-2	18
Protein	0.3-1	4-10
Lipid	0.2	1-4

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