



# **Waste to Wealth- Value Recovery from Agro-food Processing Wastes Using Biotechnology: A Review**

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## **Authors' contributions**

*This work was carried out in collaboration between all authors. Author TINE conceived, designed and supervised the study, assisted in managing the literature searches and also prepared the manuscript. Authors UEE and CO managed the literature searches. All authors read and approved the final manuscript.*

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## **ABSTRACT**

From creation, man was charged to 'increase, multiply, and subdue the earth'. Thus, man has continually sought to improve the quality of life by transforming nature to provide more food, and better living conditions for long life. Agriculture and technology are part of the tools used to accomplish this transformation and to achieve many of man's goals. Through mechanization and other tools of modern technology man cultivates crops and rears animals for his food needs, processes same through industrial activities for value addition, and carries out other sundry activities- all in a bid to dominate his environment. Major fallout of all these is the generation of wastes, with their attendant implications for the environment generally. Generation of wastes demands that measures must be taken to manage them if the unpleasant consequences of their accumulation (and these are legions) must be averted. Hence, various waste management options have been adopted over time, most of which had tended to see waste as useless entity that must be disposed off wholesomely. However, modern concepts of waste management tend to see waste from a different perspective, since what is regarded as waste may not be truly totally so, at least, from the point of view of salvageable resources entrapped therein. This is

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particularly so for agro-food wastes in which reasonable percentage of biomass form major part of what is often considered as wastes consigned to the waste bins. Fortunately, the entrapped resources are bioconvertible into useful products: animal foods and feeds, biofertilizers, industrial chemicals/raw materials, biofuels, biogas and other energy renewable alternatives, etc. With respect to these, the role of biotechnology, in resource exploitation can hardly be overstated. Thus, this paper explores wastes as a veritable resource for wealth creation, with particular focus on resources recoverable from agro-food wastes using the tool of biotechnology.

**Keywords:** *Waste to Wealth; Value Recovery; Agro-Food Wastes; Biotechnology.*

## **1. INTRODUCTION**

### **1.1 Biotechnology- Definitions and Applications**

By way of definition, biotechnology is a branch of study that deals with the application of the knowledge of biological sciences to procuring solutions to technological problems. The reverse definition is also correct, being also the application of technological solutions to biological problems. It is a field where there is an interface between various biological sciences( microbiology, biochemistry and molecular biology) and engineering sciences; techniques wherein biological process such as microorganisms or parts thereof are applied to make useful products. This blending has given rise to useful interventions in the various aspects of problems beseeching humanity as is clearly evident from the various applications of this novel technology. Among the new technologies that have appeared since the 1970s, biotechnology has attracted the most attention. It has proved capable of generating enormous wealth and influencing every significant sector of the economy. It has already substantially affected healthcare, production and processing of food, agriculture and forestry, environmental protection, and production of materials and chemicals [1].

In biotechnology, a biological material is used to realize a product in commercial scale. Biotechnology is based on many disciplines such as biochemistry, microbiology, genetics, zoology, botany, physics, chemical engineering, food engineering, etc. As a result of increasing interest in these biotechnological processes, many institutions and work groups attempt various definitions for biotechnology, each, according to their perception of it, particularly with reference to how the technology affects their sphere of activities or work. Taking cue from the definition of Karl Ereky who used this term in 1919 for the first time, Bermek [2] defined it thus: "Biotechnology is a process that raw materials are converted to new products by living organisms". European Federation of Biotechnology [3] defined biotechnology as "the integration of natural sciences and engineering in order to achieve the application of organisms, cells, parts thereof and molecular analogues for products and services". The multidisciplinary feature of biotechnology was emphasized in this description. In their report, the Organization for Economic Cooperation and Development [4] gave another definition of biotechnology. According to this report, biotechnology is the application of science and technology to living organisms, as well as parts, products and models thereof, to alter living or non-living materials for the production of knowledge, goods and services. The living organisms in this definition include microorganisms, animals and plants (particularly, the cells and enzymes of these organisms). In the description, the term "goods" expresses the products of the industries concerning food, drink, drug and biochemical substances. The term "service" mentioned above explains the treatment of environmental

pollution. Relating to the use of biotechnology for treatment of waste materials, the OECD report [4] gave a more suitable definition for biotechnology as "fermentation using bioreactors, bioprocessing, biopulping, bioleaching, biodesulphurisation, bioremediation, biofiltration and phytoremediation." Processing of wastes and prevention of environmental pollution require difficult and expensive methods. Because of that, studies are done continuously on new processes in an attempt to find a way out. Among these studies, microbiological processes are one of the most interesting topics. The objectives of these processes are the degradation of wastes and the occurrence or realization of new products. The living organisms used in this method are yeasts, bacteria, fungi and algae. The processed waste materials and products obtained by these processes are very different and they exhibit diversities from one country to another [5,6].

Biotechnology has various application fields ranging from waste treatment to medical treatment. A cleaner environment, advanced methods of diagnosis and medical treatment, better products and alternative energy resources can be considered among the benefits of biotechnology. Nowadays, environmental pollution is one of the most important problems facing all countries of the world. Biotechnology offers many treatment methods to overcome this pollution problem. In this write up, various applications of biotechnology in waste removal, processing and recovering of value from wastes are all examined, as these form part of the environmental management strategies for sustainable development. In focus here however, are agro-food processing wastes

## **1.2 Wastes—Definition, Disposal Problems and Prospects of Efficient Disposal Alternatives**

Waste can be defined as unwanted materials which are discarded from a variety of sources. Waste refers to anything considered useless, but produced by the same action that produces something useful. It could be a by-product of households, industrial, agricultural, municipal, mining, commercial, and sundry other ventures, activities or sources. When something is unwanted and no longer serves a purpose, it is generally thought of as waste and discarded. Many writers have also written on waste and therefore have different views on what waste is. Gilpin [7] defined waste as material of solid or semi solid character that the possessor no longer considers of sufficient value to retain. Jennifer and James [8] also defined waste as any material used and rejected as worthless or unwanted.

However, the word waste may have different connotations, since what one considers waste may not be waste for another person. In other words, some wastes are not totally useless. According to Odoch [9], what are usually considered as waste may no longer hold since such can be recycled to produce another product. The position of this author is corroborated by what obtains in some countries of the world, where waste recycling had become an entrenched part of waste management strategies. In my country, Nigeria for instance, quite often one sees waste scavengers with their hung-over sacks as they ransack waste heaps for all manner of things, ranging from plastics, batteries, old auto parts, other metal junks and the likes, which are ultimately sold to the parent smelting industries that convert them into essential raw materials for fabricating various plastic, metal or other equivalent wares for households and/or industrial applications or uses. Perhaps, it is in the Agricultural and food industrial sector that this is prominently evident, particularly with the advent of the search for alternative or renewable energy sources to take over from the fast depleting finite (fossil fuel) energy sources with the attendant energy crises threatening the inhabitants of the world.

Essentially, this class of wastes is biological in nature, with reasonable components of them being made up of energy-rich photosynthetic biomass entrapped.

A major problem facing most developing nations of the World is to increase agricultural production without degrading the environment. Food is a basic human need and producing enough to feed the growing population of developing nations is one of the biggest challenges facing a large proportion of nations. Hence, there should be a greater intervention in form of environment friendly science and technology in food production [10]. One of such environment friendly intervention is effective management of wastes, particularly as it concerns agricultural and food processing wastes. The quality of the total environment and health status of the inhabitants are related to the quality and quantity of wastes generated in those areas, as partly defined by the nature of activities carried out by the populace. This environment-health relationship dynamics are particularly evident in most tropical environments where various environmental media are laden with sundry pollutants most of which are often furnished by wastes. In Nigeria for instance, it is not disputable that municipal waste is the most visible and serious environmental problem, given the mountainous heaps of wastes (particularly refuse) that are common sights in greater number of her urban cities , doting the roads and disfiguring the landscape, with sundry public health implications [11].

Wastes get generated at the level of household, agricultural farms and industries including Agro-food processing industrial activities. Agricultural activities produce many types of wastes in their daily operations such as biological waste, solid waste, hazardous waste, and waste water [12]. It is important that these wastes are identified and managed properly to protect the dwellers in the community as well as the environment. Waste is directly linked to human development, both technologically and socially. The compositions of different wastes have varied over time and location, with anthropogenic activities, particularly industrial development and innovation, being directly linked to waste materials. Various waste management options have been adopted at various times by different countries, industries and organizations, and all of these have applied various levels of technologies, depending on what is available and accessible at a given time in terms of available technology and this too is defined by financial and other resource availability and level of education / knowledge of operators. Improperly managed waste due to any reason, including but not limited to faulty application of available technology would definitely produce sundry consequences for the environment generally. In these instances, terrestrial, aquatic and arboreal aspects of the environments and their indwelling species are all adversely affected from the resultant pollution. The unsavory effects of improperly managed wastes are particularly worrisome in the agricultural and food processing industry because of the peculiar nature of that industrial sector as dealing on biological items with greater potentials for spoilage- biodegradable, putreable and perishable, with tendency for odor, rat and fly nuisances; the attendant health implications of food spoilage and associated food-borne diseases, and other diseases vectored by flies, rats and other potential vectors, the presence of which are facilitated by wastes.

Apart from these, some 'bad' products or fallouts of adopted production technology in the industries form part of the issues to be contended with in terms of the kinds of wastes generated and particularly so when these are not biodegradable or environmentally friendly. As an example, agricultural mechanization and food processing technology may introduce some undesired dimension to the issue of waste production and waste management. For instance, metal cans from a food canning industry may turn out to become water-collecting receptacles or vessels for breeding of mosquitoes- the vector of malaria parasites and other

disease-causing pathogens. Based on this, it is necessary that technological evaluation activities should be expanded and more thought devoted to determining what method is needed to solve today's pressing problems connected with waste management. In this regard, attention has been mainly focused on problems connected with treatment of wastes at the end of the production line. Accordingly, it is extremely important that serious considerations must be given to the issues related to the methods used in handling, treating and disposing of wastes, since wastes dumped into streams, drainage channels, creeks, lagoons and other water impoundment points create serious environmental problems likely to produce adverse effect on air, water and soil conditions, and may constitute a nuisance to those dwelling nearby. Improper waste management may result from lack of proper education and adequate information on amount and type of waste generated, best option(s) for handling, treatment and disposal of wastes on the farms and allocation for cost of handling waste. Most of the facilities and equipment for collecting, spreading and treating waste are capital intensive, handling is also laborious and therefore the knowledge of types and quantity of waste generated is very essential.

Waste could exist either as solid, liquid or gas and the effect of each phase varies. The health implications of these wastes includes: breeding of flies which carry germs on their bodies and legs and deposit them on our food; mosquitoes (vector of malaria parasites and other disease agents) breed in stagnant water, in blocked drainages and in cans, tyres, etc that collect rain water; breeding of rats and other rodents which spread typhus, salmonella, leptospirosis, Lassa fever and other diseases; they also cause injuries by biting and spoil millions of tons of food. The refuse workers themselves also face some hazards including parasite infestation and infected cuts resulting from skin contact with refuse; others include hazards on disposal sites e.g. injuries from glass, razor blades, syringes, tissue damage or infection through respiration, ingestion or skin contact.

Traditional methods of disposing crop residues and animal droppings cannot be considered of high efficiency, and therefore present as a regular source of hazard to human and animal health, as well as representing a major source of environmental pollution, with a plethora of environmental problems. The issues associated with wastes management are numerous and if not properly handled, could result into serious environmental problems, possibly escalating into disastrous dimensions. The dangerous nature of waste and pertinent search for their solutions demanded suggestions and consequent considerations of waste minimization and recycling techniques as veritable options for agricultural and food processing wastes.

### **1.3 Agro-Food Processing Wastes: The Concept of Waste Minimization and Recycling**

The concept of waste as a material "which has no use" is changing to that of seeing waste as a resource by converting into secondary material with modification. Wastes can thus be converted into useful resources used at home or even sold for wealth. Waste recycling involves the collection of discarded materials such as husks, peels, poultry droppings, cow dung, biomass etc and processing these materials, and turning them into new products [13]. As a matter of fact, recycling helps to turn waste into other usable forms. Waste recycling is synonymous with resource recovery. It is a process of turning what has been considered as waste into useful products. The essence of recycling is to minimize the quantities of waste exposed to our environment and the consequent health hazards. According to Safeguard International Training Institute [14], waste minimization is at the top of every version of a

waste management hierarchy around the world and is considered by many to be the most important management technique to be applied to waste. The objective of waste recycling is to put what has been discarded as waste into another useful product. According to Gillian [13], there are six benefits of recycling wastes:

1. It reduces the amount of waste requiring disposal
2. It saves natural resources including non-renewable resources such as petroleum.
3. It reduces the amount of energy needed to manufacture new products
4. It reduces pollution and destruction caused while obtaining new raw materials.
5. It provides employment opportunities.
6. It helps the national economy because fewer raw materials have to be imported.

Methods of cultivating the soil, harvesting crops, raising livestock have changed with time. Today's agriculture relies on a wide range of fertilizers, fungicides, herbicides, other pesticides and hormones. The benefits of using these chemicals in modern agriculture include increase in agricultural produce and reduction in crop losses. Increase in agricultural produce leads to a corresponding increase in agricultural wastes, which have the potentials of causing environmental pollutions. Agricultural wastes are all forms of plant-derived or animal-derived materials that are considered useless either because they have no known positive economic importance or because they are not grown or raised for any specific purpose [15]. Wastes produced due to agricultural activities are classified as:

- Crop residues
- Pruning residues from trees and date palms
- Weeds and water weeds harvested from rivers, canals, and drains
- Animal droppings
- Food processing wastes.

Residue from agricultural production make up 30–60 percent of the product that is used for human consumption and animal feed [9]. Agricultural wastes are produced and generated through agricultural processes such as food manufacturing, animal rearing, household wastes from kitchens, etc. On the other hand, industrial wastes come from different industrial activities. Wastes from agricultural processes chiefly fall into solid and liquid wastes. Large quantities of vegetal wastes may be generated also as a consequence of intensive production of field crops or livestock by farmers. Over the years due to increase in population, consumerism, urbanization, industrialization, increased agricultural production and other related factors, waste generated has increased substantially. While the Wastes generated from field crops agro-food processes are richer in Lignocellulosic wastes, those produced from livestock agro-food processes are richer in Animal Residues. Let us look briefly at the nature and characteristics of both Lignocellulosic wastes and those of animal residues with a view to examining their potentials as raw materials or substrates for production of various useful products in a waste to wealth scheme.

## **1.4 Types and Reuse Potentials of Food Processing Wastes**

### **1.4.1 Lignocellulosic wastes**

Wastes in this class are composed principally of cellulose, hemicelluloses and lignin, and include cereal and vegetable wastes such as straw, bagasse, cobs, cotton husk, groundnut husk, fibrous remnants of forage grass among others. They are arguably the most abundant

agricultural wastes [16]. In general, wastes in this class are composed of nearly 50% cellulose on a dry weight basis while hemicellulose and lignin account for the balance on a nearly equal basis [17,18,19]. A cursory look at Table 1 shows the lignocellulosic composition of broad range of residues usually produced from harvesting and processing of Agricultural commodities. On a global scale the quantity of cellulosic wastes available varies with the predominant agricultural and industrial crop produced in a given society. Although lignocellulosic wastes have found significant application as sources of (heat and electric) energy, it is believed that considerable value addition may be achieved by using these wastes for animal nutrition [18,19]. Unfortunately their use in animal feeding is constrained by very low content of protein, vitamin, oil and other nutrients and limited digestibility and palatability to ruminants. However, they may be applied for animal nutrition following protein enrichment by using a variety of microorganisms like fungi and bacteria. The predominant efforts in this direction have emphasized the use of Solid Substrate Fermentation (SSF). In addition to the protein enrichment of wastes for use in ruminant nutrition, they have also been employed as principal raw materials, as a carbon source, for the fermentative production of feed-related products such as enzymes and organic acids, single cell oil, flavour compounds, etc. The use of these wastes as principal carbon and energy sources for the production of microbial biomass protein such as single cell protein (SCP) and mushrooms has also received considerable attention, and a number of topical reviews exist in the literature.

**Table 1. Lignocellulosic composition of broad range of residues usually produced from harvesting and processing of agricultural commodities**

<b>Lignocellulosic waste</b>	<b>Cellulose (wt%)</b>	<b>Hemicelluloses (wt%)</b>	<b>Lignin (wt%)</b>
Barley straw	33.8	21.9	13.8
Corn cobs	33.7	31.9	6.1
Corn stalks	35.0	16.8	7.0
Cotton stalks	58.5	14.4	21.5
Oat straw	39.4	27.1	17.5
Rice straw	36.2	19.0	9.9
Rye straw	37.6	30.5	19.0
Soya stalks	34.5	24.8	19.8
Sugarcane bagasse	40.0	27.0	10.0
Sunflower stalks	42.1	29.7	13.4
Wheat straw	32.9	24.0	8.9

Source: Nigam et al. [22]

The choice of microbial type to grow on lignocellulosic wastes depends to a large extent on the desired end product, and on whether or not a pre-treatment step is included or needed in the cultivation process. Pre-treatment processes that have been applied include steam explosion, acid, alkali, peroxide treatment, gamma irradiation, and combination of two or more of these processes as well as the usually more expensive but environmentally friendlier enzymatic treatment using a variety of cellulases, hemicellulases and ligninases. In many instances, protein enrichment of agro-food waste may be accompanied by the economic extraction of valuable biochemicals such as food/feed grade enzymes and organic acids. In most cases the production of protein enriched lignocellulosic waste has been associated with the reduction in the content of lignocellulose (associated with loss of biomass via microbial respiration as carbon dioxide). Of particular importance in the protein enrichment of lignocellulosic waste for feed use is the improvement in palatability, acceptability and digestibility of such treated wastes for ruminants [20]. This is due to the

enzymatic disintegration of the lignocellulosic structure of plant cell wall. In addition, the low technology and reduced reactor volume employed in the SSF process [21] means that the process may be easily adapted for use in less affluent farm communities.

#### **1.4.2 Animal Residues**

The accumulation of animal residues causes severe environmental pollution. The utilization of the residues other than as manure has proceeded mainly through biological and thermochemical processes. The biochemical process produces primarily sugars and alcohols, while the anaerobic fermentation produces methane. The thermochemical process involves dehydration and hydrocarbonation to yield char (carbon), oil and gaseous fuels. The greatest handicap of the thermochemical process is the large amount of moisture that needs to be removed to make the process feasible. Dried animal blood, which is sometimes used as organic fertilizer, contains about 12% nitrogen with traces of phosphorous, iron, copper and other minerals. It is often mixed with superphosphates and used as compound fertilizer [23]. The nutritive value of animal bloods from abattoirs has been reported [24] and used primarily as a source of protein [25]. Blood char is a form of activated carbon from animal blood. It serves as an adsorbent, capable of adsorbing gases many times its volume. Blood char selectively adsorbs colouring matters from solutions under specified conditions and is thus used as industrial decolourant, particularly in the sugar industry, and in gas masks [26, 27].

Animal blood has been used for the production of blood albumin [23]. It is usually obtained from coagulated animal blood. Commercially available blood albumin usually called dried blood serum is a cheap substitute for egg albumin powder. Blood albumin is often used to obtain lighter coloured protein finishes for leather and gives high lustre without altering the breathing property, unlike synthetic resin finishes that block the pores in leather. Blood albumin is soluble in water and in suitable concentrations provides highly tacky solutions that give firm adhesive properties. The substrate joints that are bonded with blood albumin based adhesives can be made water resistant by heat or formaldehyde treatment. In Nigeria, large quantities of these wastes are produced annually and are vastly underutilized, although there have been reports on the utilization of these wastes. The level of waste management and recycling in Nigeria in general are very low. This could be as a result of inadequate knowledge on waste disposal, management and recycling strategies [28]. Two actions that are required is the challenge to change personal and public view towards waste utilization and the need to provide the appropriate incentives to stimulate greater use of utilization technologies. The ignorance on how to effectively manage wastes and on the benefits that could accrue from such management of wastes has led to dumping waste products in rivers, streams, holes, seas, etc, [29]. Another problem is due to poor and unplanned waste disposal that makes rural communities unaware of the processing and reutilization of wastes [30]. Inadequate disposal management and re-utilization of waste cause environmental pollution. Improper waste disposal in rivers, streams and ponds pollutes the water by introducing bacteria or nitrate which are harmful to man and animals. Odours from animal dwellings may cause problems with neighbours and create a negative public perception [31]. The Ministry of Environment has a responsibility to enlighten the public and create awareness on the treatment and reutilization of waste, which minimizes cost and ensures the health status of citizens.

There are a number of concepts about waste management which vary in their usage between countries or regions. It is suggested that the best option for waste management is to follow the golden rule of 3 "Rs" Philosophy viz: reduce, reuse and recycle whereby the

waste generated is not only minimized but converted into an asset for reuse [32,33]. An example of this may be found in the removal dye stuffs using saw dust [34], hard wood [35], and peanut shell [36]. For this purpose, adsorption onto activated carbon appears to be the most interesting from point of view of large scale application, simple technology, and cost effectiveness [37]. These activated carbons (commercial) are usually very costly and readily unavailable and therefore can hardly be afforded in most developing countries like Nigeria due to limited availability of foreign exchange. Since agricultural wastes are available abundantly at no or low costs, it has the potential to provide a low cost industrial raw materials and adsorbent for cleaning our environment. The aim of waste recycling is therefore to extract the maximum practical benefits from it and to generate the minimum amount of waste [38]. Therefore, while there is enough information on the strategies of making waste useful for sustainable agricultural and environmental protection, there is little information available on the assessment of the economic benefits of these recycling methods.

Agricultural and food industry's refuse and wastes constitute a significant proportion of worldwide agricultural productivity. It has been variously estimated that up to 30% of global agricultural produce are left behind in the farms as residues and refuse. This is in addition to the proportion of photosynthetically produced energy that ends up as unused but potentially usable biomass [39,40]. Large volumes of wastes, both solids and liquids are also generated from the food processing industries. The problems posed by the continuing accumulation of these wastes have been compounded by the increasing concentration of food processing activities in large industries, particularly in the developed countries. Even in the less developed nations, the need to transport large quantities of food to the cities has led to increasing movement of processed food. In addition, emphasis on intensive agriculture to meet growing demand, as well as the concentration of food processing facilities in small land areas have resulted in the production of wastes in much higher concentrations than the available land space can take for disposal. The non-utilization of these vast resources constitutes significant loss of value.

Growing global demand for environmentally sustainable methods of production, pollution prevention and also economic motives have changed the way wastes and refuse are looked at. In other words, wastes have come to be seen more as resources in the wrong location and form than as a problem to be safely disposed of. Aside from their capacity to cause pollution, most food processing wastes are potentially of good quality to be recycled as raw materials for other applications and/or may be reprocessed to higher value products with relative ease. Even without any biotechnological improvement or upgrading, a wide variety of these wastes are already in use, albeit in small quantities in animal nutrition and other biotechnological processes [41]. Developing and deploying appropriate technologies for the reprocessing and reuse of these abundant energy-rich resources in human or animal feeding will go a long way in increasing resource utilization efficiency. In particular, it will help to fight protein-energy malnutrition in those areas of the world where humans and animals compete for the same sources of protein and calories. Besides reducing pressure on productivity and improving global food security, efficient utilization of wastes will help to improve environmental health particularly with respect to those wastes whose accumulation constitute significant health hazards, and whose treatment and disposal incur considerable cost penalties.

However, the idea behind waste minimization, which is the emerging face of waste management, requires that much of what is considered as waste and refuse, particularly those of considerable calorific value be seen first as valuable raw material for the production

of value added products than as waste for disposal. This approach is likely to reduce ever increasing volumes of waste meant for ultimate disposal. The fact that these wastes could become very useful substrates for the production of very much important class of products including organic fertilizers, animal feeds, drugs, industrial raw material (e.g. essential oils, enzymes, etc) is indeed a big plus both for the various industries and public health programme of environmental health and safety [42] The applications also offer an alternative waste management option for various classes of wastes, particularly household kitchen wastes of agricultural produce that is usually present in great abundance in our environment. By so doing, these wastes with great potential for environmental degradation, pollution and disease causation, are turned into raw materials for the various industries including (pharmaceutical industry), thus, becoming a veritable resource for industrial growth, with possible positive impacts of this exploitation on job and wealth creations for national economic prosperity. Added to these are the public health impacts of a safer and healthier environment likely to be secured through the indirect waste management option so offered [11,43]

## **2. FOOD PROCESSING WASTES AS VALUABLE RESOURCES FOR INDUSTRIAL AND OMESTIC APPLICATIONS**

Food processing wastes are those end products of various food processing industries that have not been recycled or used for other purposes. Food processing industries release a large amount of waste materials because they process the crude raw materials (fruits, vegetables, animals, spices, condiments, cereals or pulses) into finished products. For example, the fruits and vegetable processing industries release 50% of the weight of raw materials as waste products in the form of peels, stones or fibres. Similarly animal processing industries (e.g. slaughter houses) release a lot of waste products in the form of skins and hides, blood, fats, horns, hoofs, hairs, feathers, shells, bones, liver, intestines etc. Some of them are of much use while others are not [44]. Food wastes are also generated from catering units, enterprises, canteens and family in the process of food processing and dining.

The composition of wastes emerging from food processing factories is extremely varied and depends on both the nature of the product and the production technique employed. For example, wastes from meat processing plants will contain a high fat and protein content, whilst waste from the canning industry will contain high concentrations of sugar and starches. Also, the waste may not only differ from site to site but also vary from one time of the year to another. Furthermore, the volume and concentration of the waste material will not be constant. This may cause some problems in managing a consistent working process due to fluctuations in the nature, composition and quantity of raw materials. In general, wastes from the food processing industry have the following characteristics:

1. Large amounts of organic materials such as proteins, carbohydrates and lipids.
2. Varying amounts of suspended solids depending on the source.
3. High biochemical oxygen demand (BOD) or chemical oxygen demand (COD).

Food wastes are harmful, which seriously affect the urban environment and human health, but because of its rich organic matter and fat content, has a greater utilization value [45-49]. Being the non-product flows of raw materials whose economic values are less than the cost of collection and recovery for reuse, they are therefore discarded as wastes. However, these wastes could be considered valuable by-products if there are appropriate technical means of

reusing them and if the value of the subsequent products is to exceed the cost of reprocessing. The various wastes from different food processing industries and potential uses of food processing wastes generated from various agricultural produce are summarized below in Table 2.1 and Table 2.2 respectively.

**Table 2.1. Various wastes generated by food processing industries**

Type of Food Processing Industry	Waste Materials
Animal products	Skins, hides, blood, fats, horns, hairs, bones, liver, intestines.
Poultry processing	Skin, blood, fats, hairs, feathers, bones, liver, intestines, wings, trimmed organs.
Marine products processing	Shells, roes, trimmed parts, pincers.
Cereals and pulse processing	Husk, hull, chaff, stalks.
Fruits and vegetable processing	Skin, peels, stones, fibre, pith.
Nuts	Shells, coir, pith.
Spices and condiments	Hulls, stalks.

Source: Rao [44]

**Table 2.2. Products from Wastes generated from the Harvesting and Processing of Some Agricultural Produce.**

Agricultural produce	Residue/Wastes generated	Potential use of residue
Corn, wheat, rice	Straw, stalks, husks and cob	Animal feedstuff, fuel, silica, Furfural, compost, chemical feedstock
Cattle	Animal waste e.g blood, bone, dung	Animal glue, animal feed supplement, methane production, activated carbon, manure
Sugarcane	Bagasse	Fuel, furfural, animal feed, particle boards, biopolymers
Fruits and vegetables	Seeds, peels and husks	Animal and fish feed, fuel compost and fermentation
Oils and oil seeds	Shells, husks, fibres, sludge, presscake	Animal feed, fertilizer, fuel, activated carbon, furfural
Coconut	fibres, shell	Resins, pigments, fillers, mats, activated carbon, tanning materials.

Source: Akaranta [50].  
The lists, however, are not exhaustive.

The composition of domestic food wastes is complex and includes oil, water, fruit, vegetables, rice, fish, meat, bones and eggs and other substances mainly starch, cellulose, protein, and other organic matters. The moisture content of domestic food wastes is usually high as well as the lipid and salt contents. This makes such wastes easy to decay and ferment, and develop unpleasant smell quickly. The organic content of food wastes is also high, and as such, food wastes contain a lot of nutrients. The untreated food waste therefore is a breeding ground for, rats, houseflies, mosquitoes, other vectors and vermins. Food wastes are also sources of various pathogens such as *Salmonella* sp, *Staphylococcus aureus*, hepatitis viruses and other pathogenic microorganisms. The food industry produces large volumes of wastes, both solids and liquids, resulting from the production, preparation and consumption of food. Currently, the transportation tools of food wastes is very simple and lacks seal, thus food wastes leak during storage, collection and transportation thereby polluting the environment. The surface water and groundwater can be polluted by waste

leachate through surface runoff and osmosis. If food wastes are dumped randomly, they will occupy a lot of land space; emit unpleasant smell after decaying, producing large amounts of toxins and stench gas that pollute water and air. If poured into the sewers directly, it will cause the blockage of the sewer, affecting the city appearance and environmental sanitation seriously, and causing environmental pollution [45-49]. Thus, these wastes pose increasing disposal and potentially severe pollution problems and represent a loss of valuable biomass and nutrients. Beside their pollution and hazard aspects, in many cases however, food processing wastes might have a potential for conversion into useful products of higher value as by-product, or even as raw material for other industries, or for use as food or feed after biological treatment.

## **2.1 Agro-Food Wastes as Substrates for the Production of Different Types of Enzymes**

The utilization of agro-food residues for the production of enzymes via Solid State fermentation (SSF) has gained renewed interest from researchers as it solves solid waste disposal problem and also produce lesser waste water. Agricultural wastes present the most inexpensive and highly energy rich substrates for fermentation. In nature, solid organic substrates such as animal and plant residues, wood, crop residues, fruits, etc. undergo complex microbial degradation and transformation by various microbiological processes. Agro-industrial residues are generally considered the best substrates for the Solid State fermentation (SSF) processes for the production of enzymes. A number of such substrates have been employed for the cultivation of microorganisms to produce lots of enzymes (Table 2.3). Some of the substrates that have been used include sugar cane bagasse, wheat bran, rice bran, maize bran, gram bran, wheat straw, rice straw, rice husk, soy hull, sago hampas, grapevine trimmings dust, saw dust, corncobs, coconut coir pith, banana waste, tea waste, cassava waste, palm oil mill waste, aspen pulp, sugar beet pulp, sweet sorghum pulp, apple pomace, peanut meal, rapeseed cake, coconut oil cake, mustard oil cake, cassava flour, wheat flour, corn flour, steamed rice, steam pre-treated willow, starch, etc (51,52). Wheat bran however holds the key, and has most commonly been used in various processes.

The selection of a substrate for enzyme production in a SSF process depends upon several factors, mainly related with cost and availability of the substrate, and thus may involve screening of several agro-industrial residues. The solid substrate not only supplies the nutrients to the microbial culture growing in it but also serves as an anchorage for the cells. The substrate that provides all the needed nutrients to the microorganisms growing in it should be considered as the ideal substrate. However, some of the nutrients may be available in sub-optimal concentrations, or even absent in the substrates. In such cases, it would become necessary to supplement them externally with these nutrients. It has also been a practice to pre-treat (chemically or mechanically) some of the substrates before using them (e.g. lignocelluloses), thereby making them more easily accessible for microbial growth.

Commercial production of enzymes is generally carried out by submerged fermentation (SmF) and solid state fermentation (SSF). The physico-chemical and nutritional requirements are unique for a particular microorganism. The composition and concentration of media and fermentation conditions greatly affect the growth and production of extracellular enzymes from microorganisms. The production of amylolytic enzymes in submerged fermentation employing synthetic media have been largely exploited [53,54] but is limited by the high cost of production. In the case of Smf the abundance of water gives more control of

environmental factors such as temperature, oxygen concentration and pH and also provides ease in handling. However few reports have suggested agricultural wastes as an alternative for synthetic basal media for the production amylase [55-57]. Large scale production of enzymes would require formulation of a cost effective media, and the commercial success of amylases is linked to the utilization of starchy biomass as an industrial raw material. The most inexpensive and highly energy rich substrates for fermentation is represented by agricultural wastes. The utilization of residues for the production of enzymes has gained renewed interest from researchers for the use of SSF as it solves solid waste disposal problem and also produce lesser waste water [58]. Initially, SSF was considered to be suitable for fungi and yeast considering the low water activity but there has been continuous exploitation of bacterial cultures [58]. Several naturally occurring agricultural by-products such as wheat bran, coconut oil cake, groundnut oil cake, rice bran, wheat and paddy straw, sugar beet pulp, fruit pulps and peels, corn cobs, saw dust, maize bran, rice husk, soy hull, sago hampas, grape marc, coconut coir pith, banana waste, tea waste, cassava waste, aspen pulp, sweet sorghum pulp, apple pomace, peanut meal, cassava flour, wheat flour, corn flour, steamed rice, steam pre-treated willow, starch etc. could be used in one or the other industrial bioprocess for the production of value added products through SSF [59]. Among the above substrates amylolytic enzyme production have been carried out mainly with wheat bran, rice bran, rice husk, oil cakes, tea waste, cassava, cassava bagasse, sugarcane bagasse [60]. Banana waste, corn flour, saw-dust, soybean meal, sweet potato, potato, rice hull, sugar beet pulp have also been tried by some of the researchers. The utilization of wastes such as wheat bran, molasses bran, maize meal, millet cereal, wheat flakes, barley bran, crushed maize, corncobs and crushed wheat have been exploited for the production of alpha amylase by thermophilic fungus *Thermomyces lanuginosus* under solid state fermentation. Among the amylolytic enzymes commercial production of alpha amylase and glucoamylase utilizing agro residues are well studied and production of amylases and pullulanase have been studied to some extent. There are limited reports on the production of cyclodextrinases and research on the utilization of agro residues are yet to be explored in detail. Thus the wastes popularly employed for amylolytic enzyme production can be broadly classified as cereal brans, oil cakes and other starchy and non starchy substrates.

**Table 2.3. Spectrum of Waste substrates employed for production of various Enzymes in solid state fermentation systems**

Substrate	Enzyme
Wheat bran	B-xylosidase, $\beta$ -glucosidase, xylanase, cellulase, acid protease, $\alpha$ -amylase
Bagasse	Laccase, Mn-peroxidase, phenol oxidase, cellulase, $\beta$ - glucosidase
Apple pomace	Xylanase
Wheat straw	Xylanase, CMCase, laccase, Mn-peroxidase, aryl-alcohol oxidase
Coffee processing plant waste	Xylanase, cellulase, $\alpha$ - arabinofuranosidase, $\beta$ - xylosidase
Rice husk	Cellulase
Rice bran	Protease
Soyhull	Cellulase
Cassava waste	Xylanase, cellulose
Soybean meal	Alkaline protease
Cellulose, starch, cellulosic wastes	Cellulase, amylase, $\beta$ - glucosidase
Sugar beet pulp	Polysaccharide degrading enzymes
Ligno-cellulosic materials	Various enzymes
Tea production wastes	CMCase, xylanase, laccase

### **2.1.1 Cereals and cereal bran**

Cereals are the fruits of cultivated grasses belonging to the genus *Gramineae*. The main cereals grown are wheat, rice, corn, barley, oats, sorghum and millet. Cereal grains contain 60–70% starch [61]. They have similar structure which includes a hull (husks) and a kernel (caryopsis) and the kernel contains three components – bran, germ and endosperm. The bran is separated from cleaned and scoured cereals during milling. Among different agricultural by-products evaluated, wheat bran was found to be the best basal and standardized medium for optimal production of alpha amylase [62-64]. The strains of *Bacillus* specie and *Aspergillus* specie. AS-2 colonized well on the wheat bran based solid media and exhibited high production of  $\alpha$ -amylase and glucoamylase [65].

### **2.1.2 Corn gluten meal (CGM)**

A by-product of corn wet milling, CGM traditionally used for animal feed, was found to be a promising substrate for the production of amylase by *B. amyloliquefaciens* due to its high content of proteins ( $\geq 60\%$ ), vitamin and other minerals [66]. Agricultural raw starches such as pearl millet, rice, gram, hordium, corn and wheat starches at 1% levels were tested for the production of alpha amylase by *B. licheniformis* [67]. Barley and oat brans are chiefly composed of beta glucans. Comparatively higher production was found in the case of pearl millet, which is represented by 56–65% starch, 20–22% amylose, free sugars ranging from 2.6–2.7% and a total protein content of 8–19% [61]. Spent brewing grain was found to be a good substrate for the production of amylase by *A. oryzae* under solid-state fermentation [68]. Spent grain is the by-product of breweries left after the grain (barley, corn, wheat, rice, and other grains) is fermented and the alcoholic solution drawn off. It is normally wet, with 80 to 85% moisture content and relatively high protein content (27–30%).

A number of reports have appeared on microbial cellulase production in recent years [52]. Production cost of cellulases may be brought down by multifaceted approaches which include the use of cheap lignocellulosic substrates for fermentation production of the enzyme, and the use of cost efficient fermentation strategies like solid state fermentation (SSF). Solid state fermentation (SSF) stimulates the natural growth of microorganisms on a moist insoluble solid support in the absence (or near absence) of free water [58]. SSF offers several opportunities for processes with agro-industrial residues, thus increasing the interest on their applications. The reutilization of agro-industrial wastes for enzymes production using SSF minimizes pollution from these wastes and allows obtaining high value-added products using an economical technology. For example, Wheat straw was used for cultivating several fungal strains to produce laccase, Li-peroxidase, and Mn-peroxidase. Several authors have used bagasse also to produce other enzymes [69,70].

### **2.1.3 Grape pomace**

Grape pomace is the residue left after juice extraction from the grapes in the wine making industry. Only in Spain, over 250 million kg of this by-product (constituted by seeds, skin and stem) are generated. This material is under-exploited and most of it is generally disposed in open areas, leading to serious environmental problems. In contrast, the potential utility of this waste for value-added products by SSF is promising (high carbohydrate content with the fibre representing about 50% of the total mass). Given this composition, it has been used as substrate for the production of hydrolytic enzymes such as xylanase, exo-polygalacturonase (a type of pectinase), cellulase, etc. [71]. However, this composition changes according to season, types of grapes, weather conditions, etc., and, therefore, no reproducible enzymatic

productivities used to be achieved. In order to enhance the optimum production of some hydrolytic enzymes, orange peels have been selected as a natural source of nutrients to mix with grape pomace. Citrus peels are the main solid by-product of the citrus processing industry and constitute about 50% of fresh fruit weight. They are rich in pectin, cellulose and hemicellulose and, in addition, constitute abundantly available waste. The disposal of the fresh peels is becoming a major problem in many factories. As for other food processing wastes, various microbial transformations have been proposed for citrus peels, producing valuable products like biogas, ethanol, citric acid, chemicals, various enzymes, volatile flavouring compounds, fatty acids and microbial biomass [72].

#### **2.1.4 Sugar cane bagasse**

Sugarcane bagasse has been used as substrates for the production of various industrially important enzymes. Many microorganisms, including filamentous fungi, yeasts and bacteria, have been cultivated on this material in fermentation processes through which such important enzymes as the following were produced:

##### **2.1.4.1 Cellulase**

Amongst the various enzymes produced in SSF of bagasse, cellulases have most extensively been studied. It is well established that the hydrolysis of the lignocellulosic residues using enzyme largely depends upon the cost of the production of cellulases. Application of bagasse in SSF for this purpose appears attractive. Sharma et al. [73] and Sharma et al. [74] reported production of cellulases from different fungal strains. Roussos et al. [75] used a mixture of bagasse and wheat bran (4:1) for the production of cellulases. They suggested hydraulic pressing as a good technique to leach out the enzymes from the fermented matter. Modi et al. [76] reported higher yields of cellulase from a strain of *Streptomyces* sp. HM29 when grown on bagasse in comparison to rice straw, rye straw and corncobs. Yields were comparable with those obtained from rice bran but lower than those from wheat straw and wheat bran. Often, cultivation of two different strains as mixed culture and pre-treatment of bagasse showed desirable impact on fermentation. Gupte and Madamwar, [77] reported that production of cellulolytic enzymes under SSF by co-culturing of two fungal strains showed improved hydrolytic glucosidase activities as compared to the occasions when they were used separately. Alkali pre-treatment improved the enzyme production [77]. Similarly, Gupte and Madamwar [77] also reported higher cellulase productivity in co-culturing of a basidiomycete strain with other filamentous fungi. A mutual synergism was observed between the parent strain of *Trichoderma reesei* (LM-UC4) and the *Aspergillus phoenicis* (QM 329), resulting in enhanced combined biomass production and corresponding increase in cellulase, endo-glucanase and glucosidase activities. When co-culturing was carried out using a mutant strain of *T. reesei* LM-UC4E1, such synergism was absent, suggesting that in the hypermutation the ability for co-operative interaction with other microbes was lost. Treatment of bagasse with ammonia (80%, w/w moisture content) resulted in higher enzyme productivity [78]. When *Trichoderma reesei* LM-UC4 and its hypercellulolytic mutant LM-UC4E1 were co-cultured with *Aspergillus niger* ATCC 10864 in solid substrate fermentation on alkali-treated sugar cane bagasse for cellulolytic enzyme production, the mutant strain was more responsive to mixed culturing than the parent strain [79]. Bagasse was supplemented with either soymeal or with ammonium sulfate and urea, and fermented at 80% moisture content and 30°C. Mixed culturing produced better results with the inorganic supplement. Their study shows that mixed culturing is beneficial for the economic production of cellulases on nutritionally poor agricultural residues, without the need for supplementation with expensive organic supplements.

#### 2.1.4.2 Xylanase

Xylanase has been another enzyme produced in SSF of bagasse. Xylanases are typically important enzymes for the degradation of plant materials (hemicellulose, which comprises mainly of xylan). Xylans are formed mainly by heteropolysaccharide of a chain of 1,4 xylanopyranose units highly substituted by acetyl, arabinosyl and glucopiranosyl residues. Most of the commercially available xylanases are being produced from fungi which are active at neutral or acidic pH and their optimum temperature for activity is below 45°C. Thermophilic xylanases, which are active at alkaline conditions, have great potential for industrial applications. Jain, [80] used a thermophilic fungus for the production of extracellular xylanase on various wastes, including bagasse. Fungus grew well on untreated bagasse and enzyme titres were lower when fungus was grown on treated (alkali or acid chlorite treatment) bagasse. Acetyl esterase was produced concurrently, maximal activity being with bagasse in comparison to other substrates. Gutierrez-Correa and Tengerdy, [81] also performed xylanase production in SSF using bagasse. They co-cultured *T. reesei* and *A. niger* or *A. phoenicis* and achieved high xylanase titres (2,600–2,800 IU/g dry wt). Sugar cane bagasse was chemically treated to generate different bagasse samples with varying quantities of lignin and hemicellulose, keeping the cellulose fraction intact. These bagasse samples were evaluated for the production of cellulase and xylanases enzymes by *Penicillium janthinellum* NCIM 1171 and *Trichoderma viride* NCIM 1051 in the production medium [82].

#### 2.1.4.3 Amylase

Studies by Rajagopalan and Krishnan, [83] showed that sugar cane bagasse hydrolysate (SBH) can be used for amylase production. Utilization of sugar cane bagasse has not been possible for amylase production by *Bacillus* sp. and there is no previous report for the production of amylase from *Bacillus* sp. in submerged or solid state fermentation. This is due to the fact that hydrolysis of sugar cane bagasse forms simple sugars primarily glucose, xylose and arabinose that repress amylase synthesis through catabolic repression. A new isolate of *Bacillus subtilis* KCC103 showed absence of repression by glucose during amylase synthesis. The level of amylase produced in sugar cane bagasse hydrolysate medium was equivalent to that in starch medium, therefore replacement of starch by SBH in production medium is highly feasible to produce amylase at low cost.

#### 2.1.4.4 Inulinase

Optimization of inulinase production by *Kluyveromyces marxianus* NRRL Y-7571 using sugarcane bagasse as substrate was studied by Marcio et al., [84]. The best fermentation conditions found after optimization was 36°C and 20% of corn steep liquor, which yielded about 390 Ug<sup>-1</sup>. Maximum productivity was 3.34 Ug<sup>-1</sup> h<sup>-1</sup>. Sugarcane bagasse seems to present a great nutritional potential for growth of *K. marxianus* NRRL Y-7571 and production of inulinase.

#### 2.1.4.5 Lipase

The use of solid state fermentation for the production of thermostable lipases is an interesting alternative to the valorization of bagasse and olive oil cake. Lipase production could be optimized by adding the appropriate precursors found in olive oil cake. Olive oil cake and sugar cane bagasse were used for lipase production using thermostable fungal cultures of *Rhizomucor pusillus* and *Rhizopus rhizopodiformis* [85]. However, the mixture of

olive oil cake and sugarcane bagasse, 50% each, increased the lipase activity compared to that obtained by *Rhizopus rhizophodiformis* and *Rhizomucor pusillus*, respectively.

### **2.1.5 Other Fruit Wastes**

Bacteria, yeast, and fungi have been cultivated under both submerged (SmF) and solid state fermentation (SSF) on orange waste, apple pomace and grape pomace for different purposes.

The most important area of citrus wastes and apple pomace utilization is the production of enzymes, especially pectinolytic ones. Pectinolytic enzymes or pectinases are a heterogeneous group of related enzymes that hydrolyze the pectic substances. Pectinolytic enzymes are of significant importance in the current biotechnological era with their all embracing applications in fruit juice extraction and its clarification, scouring of cotton, degumming of plant fibers, waste water treatment, vegetable oil extraction, tea and coffee fermentations, bleaching of paper, in poultry feed additives and in the alcoholic beverages and food industries [86].

Both SmF and SSF conditions were evaluated by several researchers for the production of the above mentioned enzymes using citrus wastes or apple pomace as carbon sources. Bacteria, yeasts, and fungi under both SmF and SSF conditions were able to produce pectinolytic enzymes using citrus wastes [72,87-93] or apple pomace [94] as carbon sources.

Apart from pectinolytic enzymes, several other enzymatic activities have been produced on apple pomace namely  $\beta$ -fructofuranosidase [95], xylanase [96],  $\beta$ -glucosidase [97], manganese-dependent peroxidase and cellulase [96]. Citrus waste on the other hand has been used for the production of  $\alpha$ -amylase, neutral and alkaline proteases [98], xylanase [72] and cellulose [96]. In literature there are a few reports on grape pomace utilization for the production of enzymes, namely pectinase, cellulase and xylanase [71,99] by different *Aspergillus* species.

In addition to solid state fermentation (SSF), commercial production of enzymes is also carried out by submerged fermentation (SmF). The composition and concentration of media and fermentation conditions greatly affect the growth and production of extracellular enzymes from microorganisms. The physico-chemical and nutritional requirements are unique for a particular microorganism. The production of amylolytic enzymes in submerged fermentation employing synthetic media have been largely exploited [53,54] but is limited by the high cost of production. In the case of Smf, the abundance of water gives more control of environmental factors such as temperature, oxygen concentration and pH, and also provides ease in handling. Again, as is also the case with SSF, some reports have suggested agricultural wastes as an alternative for synthetic basal media for the production of amylase [55-57]. Large scale production of enzymes would require formulation of a cost effective media. Thus for commercial success in amylolytic enzymes (amylases) production, the wastes popularly employed as industrial raw material are broadly classified as cereal brans, oil cakes and other starchy and non starchy biomass substrates.

In many industrial processes, enzymes provide a viable alternative to chemical hydrolysis due to its high specificity, besides being environmental friendly [100]. Among enzymes, hydrolytic enzymes have prevalent applications in textile industry, the production of juices and fruit extracts, the pulp and paper, and animal feed industries etc. These enzymes

degrade polysaccharides in the plant cellular walls like celluloses, hemicelluloses and pectins. In most cases, agro-industrial wastes used to produce these enzymes by SSF do not possess all the necessary nutrients for this purpose, or maybe, they are available in suboptimal concentrations. In these cases, the substrate must be supplemented to stimulate or improve the enzyme production by adding extra carbon sources or extra nitrogen sources [101]. Supplementation can also be carried out with the adjustment of the initial moisture content of the residue using a solution containing mineral salts or mixing the solid with another residue [101].

## **2.2 Agro-Food Wastes as Substrates in Citric Acid and Propionic Acid Production**

Citric acid (2-hydroxy-1,2,3-propanetricarboxylic acid) is a natural constituent and common metabolite of plants and animals. Being the most versatile and widely used organic acid, Citric acid is an important commercial product with a global production reaching 840,000 tons per year. Citric acid has a long list of applications in industrial sectors. It is used in the food, beverage, pharmaceutical, chemical, cosmetic and other industries for applications such as acidulation, chelation, emulsifiers, antioxidation, flavour enhancement, preservation, and plasticizer and as a synergistic agent [102-107]. The food industry utilizes about 70% of the total production of citric acid [108], the pharmaceutical industry consumes 12% and the rest 18% has market for other applications. Its rising demand is subsequently causing an increase in global production. Citric acid is mainly used in the food industry because of its pleasant acidic taste and its high solubility in water. It is accepted worldwide as "GRAS" (generally recognized as safe) and is approved by the joint FAO/WHO Expert Committee on Food Additives.

Citric acid was produced by *Mucor* and *Penicillium* species as fungal metabolite in media limited in phosphate. The presence of citric acid was detected as a by-product of calcium oxalate produced by a culture of *Penicillium glaucum*. A great number of problems had to be overcome, before an effective fermentation process could be used commercially [109]. Other investigations showed the isolation of two varieties of fungi belonging to genus *Citromyces* (namely *Penicillium*). Initially, for the production of citric acid, microorganisms were cultivated in surface culture. At present over 99% of the world's output of citric acid is produced using the process of microbial fermentation. The optimization of citric acid production was recently studied by [110] and [111]. Currently citric acid is produced by fermentation-technology, using the filamentous fungus *Aspergillus niger* mainly through surface (solid or liquid) and submerged fermentation of starch or sucrose-based media [45, 105]. The filamentous fungus *Aspergillus niger* is the most commonly used microorganism for citric acid production, although several other microorganisms are used, which include various *Aspergillus* sp., yeasts such as *Candida tropicalis*, *C. oleophila*, *C. guilliermondii*, *C. citroformans* and *Hansenula anomala* [104,102,106,112,113]. However, most of them may not be able to produce citric acid on a commercial scale. Citric acid is produced commercially either by submerged fermentation, surface fermentation or solid state fermentation employing various carbohydrate sources [107,113-117].

As substrates, several raw materials such as hydrocarbons, starchy materials and molasses, have been employed for commercial submerged citric acid production (Table 2.4) [45,104, 106,118 -122], although citric acid is mostly produced from starch or sucrose based medium using submerged fermentation. Generally, citric acid is produced by fermentation using inexpensive raw material [123], including crude natural products, such as hydrolysate starch, sugar cane broth and by-products like sugar cane and beet molasses [113,124].

Molasses is preferably used as the source of sugar for microbial production of citric acid due to its relatively low cost and high sugar content (40-55%) [104]. Since it is a by-product of sugar refining, the quality of molasses varies considerably. Not all types are suitable for citric acid production. The molasses composition depends on various factors such as the variety of beet and cane, methods of cultivation, conditions of storage and handling (transport, temperature variations). Both beet and cane molasses are suitable for citric acid production, however, beet molasses is preferred to sugarcane due to its lower content of trace metals, supplying better production yields than cane molasses, but there are considerable yield variations within each type. In the case of cane molasses, generally it contains some metals (iron, calcium, manganese, zinc) which retard citric acid synthesis and therefore requires some pretreatment for the reduction of these metals. Palmra jiggery, sugar syrup from the palmyra palm is a novel substrate for increasing the yield of citric acid production [125]. The addition of phytate (an important plant constituent) at the beginning of incubation of beet molasses results in about 3-fold increase in citric acid accumulation [126].

A variety of agro-industrial residues and by-products has also been investigated with solid-state fermentation techniques for their potential to be used as substrates for citric acid production such as cassava begasse, coffee husk, wheat bran, apple pomace, pineapple waste, kiwi fruit peel, grape pomace, citrus waste, etc. (Table 2.4). [106-107,127-131,114-116]. Molasses, carob pod extract, rape seed oil, corncobs, apple and grape pomace, kiwifruit peel, mandarin orange and brewery wastes have all been used as substrates in citric acid production [132,133]. It is necessary to use inexpensive and readily available raw materials in industrial production processes. From this point of view, large volumes of starchy materials are suitable substrates for the production of citric acid, since they are cheap and renewable [134]. This has offered an increasing avenue towards efficient utilization of and value-addition to these residues, besides being a form of reducing environmental concerns. These residues are very well adapted to solid state cultures due to their cellulosic and starchy nature. A cost reduction in citric acid production can be achieved by using less expensive substrates, such as industrial waste products mentioned. For instance, in order to achieve economic development, focus has shifted to the industrial application of cassava for value addition. Industrial processing of cassava tubers is mainly done to isolate flour and starch. Processing for flour generates solid-residues including brown peel, inner peel, unusable roots, crude bran and bagasse. Cassava bagasse is a fibrous residue from the extraction process, which is generated during the separation stage. This bagasse contains about 40-70% starch that physically could not be extracted. It has a large absorption capacity and may hold up to 70% moisture. This waste can be used for citric acid production along with two other potential substrates, sugarcane bagasse and coffee-husk. Citric acid production from cassava bagasse can be carried out using a culture of *Aspergillus niger* NRRL 2001 in solid state fermentation [59,102].

Industrial production of Citric acid can be carried out in three different ways: by submerged fermentation, surface fermentation and solid-state fermentation or Koji process [107,113-116]. All of these methods require raw material and inoculums preparation. In industrial citric fermentation, the large-scale spore production is made by using appropriate means and conditions such as direct inoculation in the production fermenter. Sometimes it is necessary to remove the remaining minerals in the raw material and add other nutrients such as phosphorous, magnesium and nitrogen for development of the mycelium and a good production of the citric acid. Of these methods however, production by fermentation is the most economical and widely used method of obtaining this product. More than 90% of the citric acid produced in the world is obtained by fermentation, which has the following advantages in its favour: operations are simple and stable, the plant is generally less

complicated and needs less sophisticated control systems, technical skills required are lower, energy consumption is lower and frequent power failures do not critically affect the functioning of the plant. The production process via fermentation can be divided into three phases- preparation and inoculation of the raw material, fermentation proper, and recovery of the product.

**Table 2.4. Various Agro-Food wastes Used as Substrates for citric acid production using submerged and semi solid fermentation method**

Raw material	Strain of Organism Used for Fermentation	Citric acid yield (kg/m <sup>3</sup> )
Beet molasses	A. niger ATTC 9142	109
Black strap molasses	A. niger GCM 7	86
Brewery wastes	A. niger ATTC 9142	19
Cane molasses	A. niger GCMC-7	113.6
Carob pod	A. niger ATCC 9142	264
Carob pod extract	A. niger	86
Hydrolysate starch	A. niger UE-1	74
Wood hemicelluloses	A. niger IMI-41874	27
Pineapple waste	A. niger ATCC 1015	132
Carrot waste	A. niger NRRL 2270	29
Cassava residue	A. niger CFTRI30	234
Cassava bagasse	A. niger LPB-21	260
Coffee husk	A. niger CFTRI 30	150
Food wastes	A. niger UV60	45.5
Grape pomace	A. niger NRRL 2001	413
Kiwifruit peel	A. niger NRRL 567	100
Rice bran	A. niger CFTRI 30	127
De-oiled rice bran	A. niger CFTRI30	92
Wheat bran	A. niger CFTRI 30	85
Apple pomace	A. niger NRRL 328	798
Apple pomace	A. niger NRRL 2270	816
Apple pomace	A. niger NRRL 567	883
Grape pomace	A. niger NRRL 567	600
Sugar cane bagasse	A. niger CFTRI 30	174
Corncob	A. niger NRRL 2270	603.5

Wheat wastes, rich with starch such as undersized semolina has also been used for citric acid production. Approximate composition of undersized semolina is 13–15 % moisture, 63–64 % starch, 11.9–13.2 % protein, 0.15–0.33 % cellulose, 1.7–2.5 % fat, 1.5–2.0 % sugar and 0.67–0.95 % minerals. Undersized semolina is generally produced during the production of macaroni from durum wheat (*Triticum durum*). About 56 000 tonnes per year of undersized semolina is produced as a waste product in Turkey, which is a cheap industrial waste of macaroni factories. This waste has been found suitable for the production of citric acid [135]. Majumder et al. [136] studied the production of citric acid from pumpkin and cane molasses using gamma ray induced mutant strains of *Aspergillus niger* under surface culture conditions. Citric acid production was found to be different with the various fermentation media by the *A. niger* strains. It was found to increase with increase in fermentation period and maximum citric acid levels were obtained after 13 days. They obtained the highest citric acid levels in a mixed fermentation medium comprising pumpkin and cane molasses. Citric

acid performs many functions and therefore finds various applications in many industrial sectors (Table 2.5)

**Table 2.5. Some Functions and industrial applications of citric acid**

Functions of Citric Acid	Industrial Sectoral Application
Tartness; complementary flavours, effective antimicrobial preservatives, pH adjuster.	Soft drinks, canned fruit juices, bottled beverages
Tartness, produces dark colour in hard sugar candies, acidulant, restricts sucrose inversion pH adjuster, antioxidant, metallic ion chelator, buffering agent	Confectioneries
Emulsifier, acidifying agent, antioxidant	Cosmetics
Lowers pH to inactivate enzymes, protects, protects ascorbic acid by inactivating trace metals	Dairy products (ice cream and cheese) Frozen foods
Removes metal oxides from surface of ferrous and non-ferrous metals for preparational and operational clearing of iron and copper oxides	Purification of metal oxides
Effervescent in powders and tablets in combination with bicarbonates provides rapid dissolution of active ingredients, mild astringent formulation, anticoagulant.	Pharmaceuticals

Source: Pandey et al. [102]

Propionic acid (PA), on other hand, is widely used as additive in animal feed and also in the manufacturing of cellulose-based plastics, herbicides, and perfumes. Salts of propionic acid are used as preservative in food. PA is mainly produced by chemical synthesis. Nowadays, PA production by fermentation of low-cost industrial wastes or renewable sources has been an interesting alternative [137]. Attempts have been made for the Production of PA by *Propionibacterium acidipropionici* ATCC 4965 using a basal medium with sugarcane molasses (BMSM), glycerol or lactate (BML) in small batch fermentation at 30 and 36°C. Bacterial growth was carried out under low dissolved oxygen concentration and without pH control. Results indicated that *P. acidipropionici* produced more biomass in BMSM than in other media at 30°C ( $7.55 \text{ g l}^{-1}$ ) as well as at 36°C ( $3.71 \text{ g l}^{-1}$ ). PA and biomass production were higher at 30°C than at 36°C in all cases studied. The best productivity was obtained by using BML ( $0.113 \text{ g l}^{-1} \text{ h}^{-1}$ ), although the yielding of this metabolite was higher when using glycerol as carbon source ( $0.724 \text{ g g}^{-1}$ ) because there was no detection of acetic acid.

### 2.3 Agro-Food Wastes As Substrates for the Production of Microbial Biomass (Single Cell Proteins)

Single cell proteins are the dried cells of microorganisms (such as yeast) that could be grown in large-scale culture systems for use as protein for human or animal consumption [138]. Microorganisms have been used for the synthesis of high protein products like cheese and fermented soybean products. They have natural ability to convert low protein organic mass into high protein organic products by various processes. This ability of microorganisms is being used to fight the problems of malnutrition and poor protein diet in third world countries, and to manufacture protein-rich food for animals. One of the most important examples is Single Cell Protein (SCP).

Research on Single Cell Protein Technology started a century ago when Max Delbrück and his colleagues found out the high value of surplus brewer's yeast as a feeding supplement for animals. During World War I, Single Cell Protein Technology proved to be more than useful as Germany used it extensively, and more than half of its imported protein sources were replaced by yeast. In 1919, Sak in Denmark and Hayduck in Germany invented a method named, "Zulaufverfahren" in which sugar solution was fed to an aerated suspension of yeast instead of adding yeast to diluted sugar solution. After the end of World War I, interest of Germany in fodder yeast declined, but got revived in 1936 by the 'Heeresverwaltung', when both brewer's yeast and other varieties of yeast (especially mass cultured) were used as supplement for human and animals. In post war period, problems of humanity were highlighted and a number of international organizations emerged for this task under the umbrella of United Nations. One such organization was The Food and Agriculture Organization of the United Nations (FAO), which emphasized on hunger and malnutrition problems of the world in 1960, introducing with it the concept of protein gap, showing that 25% of the world population had a deficiency of protein intake in their diet. It was also feared that agricultural production would fail to meet the increasing demands of food by humanity. By the mid 60's, almost quarter of a million tons of food yeast were being produced in different parts of the world, and by 1970, Soviet Union alone produced some 900,000 tons of food and fodder yeast to compensate agricultural protein production deficiency. Advances in Biotechnology techniques has participated in the development of SCP technology and helped in improving its quality and use of different organisms along with yeast for the production of SCP [139].

### **2.3.1 Fruit and vegetable wastes as substrates for microbial biomass (Single Cell Protein) production**

Nowadays people are becoming health conscious and consume large quantities of fruits and fruit juices leading to the accumulation of fruit wastes. The disposal of wastes is a serious problem and their deposition poses health hazard to man and animals. These wastes can be used as a substrate for the growth of food fungi such as *Aspergillus oryzae*, *Rhizopus oligosporus* and several species of *Agaricus* and *Morchella*, the sources of protein, which may be utilized as a feed supplement for domestic animals and cattle and if found suitable for human consumption [140]. A variety of fruit wastes have been used as substrates for the production of SCP by various researchers. Kamel [141] reported the use of dates as a potential substrate for the production of single cell protein. Sweet orange residues have been used for SCP production by Nwabueze and Oguntiemein [142]. Sweet potato residue has been used for SCP production by Yang [143]. Rahmat *et al.* [144] used apple pomace for the production of single cell protein from *Kloeckera apiculata* and *Candida utilis* so as to improve stock feed. Pineapple cannery effluent has been utilized for SCP production by Nigam [145]. Essien *et al.* [146] utilized banana peel as a substrate for mould growth and biomass production. Khan *et al.* [140] investigated the production of fungal single cell protein using *Rhizopus oligosporus* grown on fruit wastes. The study revealed that papaya fruit waste generates highest amount of protein per 100g of substrate used, followed by cucumber peelings, pomegranate rind, pineapple fruit skin and watermelon skin respectively with 59.5 mg, 57.3 mg, 51.6 mg, 48.0 mg and 43.2 mg crude protein respectively. Production of fungal biomass on fruit and other agricultural wastes shall not only minimize loads of pollutants but at the same time the malnourished people can have protein supplement at an affordable cost. The degree of fungal biomass growth depends on the type of substrate used. A comparative study of fruit wastes revealed that banana skin generates highest amount of protein per 100 g of substrate used, followed by that of rind of pomegranate, apple waste, mango waste and sweet orange peel. The amount of crude protein estimated

by Kjeldahl Method was found to be 58.62%, 54.28%, 50.86%, 39.98% and 26.26% respectively. In their own study, Maragatham and Panneerselvam [138] used extracts of papaya fruit as substrate for single cell protein production using *Saccharomyces cerevisiae*. They extracted 500 g of papaya fruit with different volumes of sterile distilled water and found that extraction with 200ml of sterile distilled water sustained highest cell growth. Biochemical analysis of dry biomass revealed the following composition: 34.0% protein, 40.0%, saccharide, 0.003% lipids, 9.54% moisture and 0.14% total ash. Nutrition found in papaya fruit extract were 9.6% saccharide, 0.2% crude protein and 7.0% total soluble sugars. Thus, fruit and vegetable wastes should be exploited properly as a substrate for the production of cellular biomass for edible or food fungi instead of dumping them on unauthorized places such as the roads, drains and water bodies. The edible or food fungi (SCP) so formed can be used as animal feed supplement and if suitable, for human consumption as well with least expenditure of money.

### **2.3.2 Animal Wastes as Substrates for Microbial Biomass (Single Cell Protein)**

A slightly different approach to the valorization of animal and fishery waste is the hydrolysis and conversion of wastes to single cell protein. Horn et al., [147] used hydrolysate of cord viscera which constitutes about 17% of the fish biomass to grow *Lactobacillus* spp. and demonstrated that the medium so formulated was as effective as commercial peptone based media used in the cultivation of the organism. This underscores the potential for the use of fishery waste of this kind for the cultivation of even fastidious organisms for the production of microbial biomass. Kuhn et al. [148] fed microbial biomass produced from fish effluent to shrimps and demonstrated that the process improved the economics of shrimp production. In addition, the process led to the effective treatment of the resulting effluent. Single cell protein production for feed use has been achieved by cultivation of organisms on ram horn hydrolysate [149,150], poultry process waste [151] and acid hydrolysed shrimp waste [152]. Composted fish waste has been used for the production of *Scytalidium aciabphilum* biomass in submerged fermentation with good protein yield for animal feeding. Amar et al. [153] also employed bacterial digestion of fish waste to produce feed for the production of Indian white prawn and in the process achieved both treatment and reuse of the fish waste. Schneider et al. [154] produced protein enriched bacterial biomass for animal feed use from a suspended growth process using aquaculture waste and in the process achieved treatment of a particularly recalcitrant waste stream. Viera et al. [155] used microalgae to treat fish pond waste water effluent, and demonstrated that the protein rich algal biomass could be used as feed for the production of abalone.

## **2.4 Food Processing wastes As Substrates for Production of Animal Feeds**

Various food processing industries produce a lot of wastes which can be of use in the production of animal feed. Notable amongst the processing factories are the palm oil/vegetable oil industry, cereal/grain processing plants as well as factories that process legumes. In the palm oil processing plants, after the extraction of palm oil from the palm fruit, the palm nuts are cracked to obtain the palm kernels. The latter are crushed and pressed or extracted to recover the palm kernel oil. The palm kernel cake (PKC) is usually discarded as waste. However, these days, PKC has found use in animal feed formulation, especially for the poultry. The cake is formulated with other supplements and given to birds as feed. Studies have shown that birds fed with such feeds have remarkably improved carcass weight. Soya bean cake which is obtained after extraction of the soya bean milk has also found use in the formulation of poultry feed. Cereals/grains milling factories generate a lot of wastes in the form of husks and chaff. These are further processed into animal feed for

poultry. Groundnut cake is obtained from the groundnut processing industry during the production of groundnut oil. After extraction of the oil the resultant cake is taken up by formulators of animal feed for the production of poultry feed. Supplementation of animal feed with such waste increases the nutritional base of the feed. Groundnut cake is rich in proteins and other vital nutrients required by the birds for proper growth. Animal products processing industries also generate large amounts of bones after deboning of meats. The bones are taken up, crushed and added to animal feeds to increase the calcium content of the feeds. Such feeds are used in the poultry especially for layers in order to strengthen the shells of eggs produced from such birds. Shells generated from marine products processing are also treated in the same way and used in animal feed formulation. The pig farms make use of food waste, which are mostly located in the suburban and rural areas. Most domestic wastes arising from homes, restaurants and hotels as well as other places involved in large scale production of food are usually collected by owners of piggeries and used as feed for the pigs. Such wastes will include fruits and vegetable wastes, solid and even semi solid food wastes. This means offers a very good avenue of disposing domestic wastes of this nature.

#### **2.4.1 Animal Food & Feeds From Slaughter House Wastes and Manure**

Large scale production of animal products including meat and poultry, and the processing and packaging of these in large scale facilities has resulted in the generation of large volumes of wastes including blood, feather, hoofs, horns, poultry intestines among others. The worldwide production of chicken intestine runs into several million tons [156]. Often these wastes are treated on site for disposal, resulting in considerable loss of otherwise useful biomass. The high protein content of these wastes makes them attractive for use in animal nutrition. However, reprocessing them for use in animal nutrition can be a considerable challenge due to their ease of spoilage. Consequently, techniques applied to these wastes are essentially preservative. Fermentative ensiling has been studied extensively as an economical process to reprocess these wastes for use as ingredients in animal feeds as alternative to the conventional, but more expensive fish and soy meal. As animal offals are poor in carbohydrates, preservation is usually effected following addition of fermentable sugars [156]. Ensiling of animal wastes also has the advantage of causing reduction in the level of pathogenic organisms present in the wastes.

Reuse of poultry litter and manure for animal feeding has also attracted considerable attention due to the inefficiency of feed utilization by poultry. Pure and mixed culture lactic acidification and silage type reactions have been variously applied to improve the protein content and preservation quality and improve the smell of poultry manure for use in the production of poultry layers, beef cattle and pigs without impairing performance [157,158]. The process is energy efficient and has enabled the use of much higher concentration of animal waste in feed formulation than could be achieved with chemically or physically modified litter and offal [159]. Lactic acidification of animal wastes is particularly interesting in the tropics where the process can be very rapid and the storage life of this form of waste can be very short, with putrefaction starting only a few hours following the collection of offal and litter. Lactic acidification also leads to rapid elimination of spoilage and pathogenic organisms [156]. A slight decline in protein content of the waste (if it happens) can be compensated for by the fact of reuse of the waste, rather than incurring cost penalties in its disposal. Long term preservation by lactic acidification of slaughter house sludge for use in animal feeding has been demonstrated in processes that also achieve rapid inactivation of potential pathogens and spoilage organisms [159-161]. As regulatory control of sludge disposal tightens, the process could play pivotal roles in waste minimization during animal production. A slightly different approach to the protein enrichment of (poultry) manure was

reviewed by El Boushy [162], which involves the use of house fly pupae as the protein enriching principle. Although this technology appears promising for the protein enrichment/extraction from poultry manure and other protein rich wastes, the necessity for sterilization at elevated temperature and its attendant energy cost may be a disincentive for the use of this technology.

#### **2.4.2 Animal Feeds From Fish and Fisheries processing Wastes**

In the fish and fisheries industries including shrimp and crustacean processing, large amounts of wastes including rejects, discards and by-products, are produced worldwide. It is estimated that up to 30% of the total landings in the fisheries industries are considered as underutilized, by-catch and unconventional or unexploited [163-165]. It has also been estimated that over 32 million tons of fish wastes accumulate annually from the processing of fish [166]. Although a proportion of these get reprocessed into fish meal, oil and cake, several tons end up as waste requiring disposal. Arvanitoyannis and Kassaveti [167] and Arvanitoyannis and Ladas [168] provide current reviews on the environmental position of fishery and meat wastes, and management processes that are being considered for handling these, particularly within the context of the European Union where several directives and legislations aim to control their disposal exist. A prominent approach to the valorization of these wastes involves their biotechnological reprocessing (particularly by ensiling) for use in animal nutrition. The ensiling of fish and shrimp wastes for use in fishery, as well as other animal nutrition, is receiving considerable attention and several studies have reported on the optimization of processes for the reuse of these wastes. In addition to using the ensiled feed for fish culture the processes lead to effective management of the waste. De Arruda et al., [169] have shown that the use of ensiled fish waste in fish feeding can significantly improve the economics of fish production, considering that feeding accounts for up to 60% of cost. Dong et al., [170] and Ngoan et al., [171] used ensiled shrimp waste to replace soy meal in the feed of duck and pigs with comparable efficiency. Coello et al., [172] optimized fish waste ensiling for the production of L-lysine using *Corynebacterium glutamicum*. The lactic preservation of these wastes by ensiling alone or with a variety of straw, forage and molasses has been shown to increase storage life of the product and increase acceptability, intake and digestibility by poultry, cattle, fish and other animals with the possibility of the silage serving a probiotic function [173-176].

#### **2.4.3 Animal Food &Feeds from Protein Enrichments of Processing Wastes of Cassava, Cocoyam, Potatoes and Other Roots and Tuber Crops**

Root and tuber crops including cassava, potato, cocoyams and yams are the principal sources of calories in many countries. The processing of these crops for human nutrition often results in the generation and disposal of several tonnes of carbohydrate rich wastes. Cassava, which is acknowledged to be a most important source of calories for large populations in the tropics, ranks as the world's sixth most important food crop [177]. Besides its significant place in tropical and global food security, cassava has recently become recognized as an industrial crop in many countries where it is playing significant roles in animal nutrition and supply of industrial starch [178].

Estimates vary considerably, but in the processing of cassava into food and starch, waste biomass may account for up to 30% of total produce [179]. Cassava wastes are very toxic due to the disproportionate partitioning of cyanogenic glycoside into the waste. As a result, without treatment, the waste can only be used in limited quantities in animal nutrition, while the capacity of cassava waste to cause pollution limits the disposal of the waste to land.

Apart from the problem of toxicity, use of the waste in animal nutrition is also constrained by its limited protein content. Yet cassava waste remains a valuable resource which if widely used in animal nutrition can reduce pressure on food crops. In order to achieve the reuse of cassava wastes, a number of processing methods have been reported which result in detoxification and improvement in the protein content of the waste thereby converting this strong environmental pollutant to a value added product. A number of processes have been implemented using cassava waste alone or in combination with other waste types, including poultry droppings to achieve reprocessing and protein enrichment of wastes. Organisms that have been employed in the protein enrichment and detoxification of cassava process wastes for use in ruminant nutrition include *Aspergillus* spp, *Trichoderma* spp. as well as a variety of bacteria, yeasts and ruminal microflora [180-182]. The carbohydrate content of cassava waste has also been variously exploited for the production of various food additives and ingredients, including citric acid and lactic acid [183-185]. Although room exists for improvement of the protein content of the product, it is interesting that by the application of relatively inexpensive solid substrate processes cassava waste could be converted into useful products rather than being disposed of by expensive waste treatment strategies.

Another equally important root tuber in this regard is Cocoyams (*Colocasia* and *Xanthosoma* spp). According to Onwueme and Charles [186], Cocoyams are widely cultivated for food in West Africa, Asia and the Oceania, with Nigeria, China and Ghana leading in world production. In 1999, worldwide production of cocoyams topped 6.5 million tones with Africa producing over half of the total [187]. In producing countries, cocoyams account for a significant proportion of the total energy intake, and this varies from about 7% in Ghana to about 18% in parts of the Oceania [188]. The processing of cocoyams to food and starch is associated with the generation of vast quantities of waste and residue that account for a significant proportion of the entire cocoyam produce [189]. The preservation and reuse of these vast wastes in animal nutrition will enhance food security in areas where cocoyams are abundant. Duru and Uma [190,191] have demonstrated the potential of using SSF to achieve over 50% increase in the protein content of cocoyam process waste using *Aspergillus oryzae*. The protein enriched waste could be used for the feeding of both ruminants and monogastric animals.

Similarly, Potatoes and Sweet Potatoes are other energy-rich root tubers widely consumed across the globe with potentials for generating reasonable amounts of process pulp and wastes. Gelinas and Barrette [192] employed *Candida utilis* to improve the protein content of waste potato starch from a chip manufacturing facility. Up to 11% protein was accumulated in a process that yielded 8% yeast protein in a submerged fermentation. In a process that mixed sweet potato and sugar cane, Rodriguez et al., [193] achieved improvement in the protein content of waste digest. Other processes in which potato process pulp and waste water, and sweet potato wastes have been reprocessed for protein enrichment including production of food and feed grade SCP have been reported [194-197]. The processes have been operated as SSF reactions, silage and as submerged fermentations.

#### **2.4.4 Animal Food & Feeds from Protein Enrichment of Fruit and Vegetable Wastes**

##### **2.4.4.1 Fruit Wastes**

Growing international production and marketing of fruits has led to increasing accumulation of fruit wastes such as citrus pulp, seeds and peels, grape pomace among others [198,199]. Disposal of these wastes can be a major cost component of fruit production since

they may account for up to 50% by weight of fruits [200,201]. These wastes are very high in cellulosic materials (cellulose and hemicelluloses), but low in lignin, making them potentially good feed sources for ruminants and promising substrates for the production of microbial protein. In countries with inadequate supplies of conventional ruminant feeds the use of fruit industry waste can impact quite positively on the supply of feed for animal nutrition while reducing environmental pollution. Unfortunately, fruit wastes have only minimal protein content which limits their value in animal nutrition. So, exploitation of these wastes in animal nutrition will depend on the deployment of processes for their protein enrichment by biotechnological means [202,203,198,204]. Fermentative processes in both SSF and slurries employing both filamentous and unicellular microorganisms have been employed for the protein enrichment of a variety of fruit industry wastes for animal feed use [205,200,91,206,207]. Up to 50% protein enrichment of apple pomace using a combination of *Candida utilis* and *Pleurotus ostreatus* has been reported [208] and these wastes have also been employed to produce food and feed grade Single cell protein. Protein enrichment and detoxification of coffee pulp for animal feed use has been reported [209]. Sunita and Rao, [210] used mango processing waste to produce blue green algal biomass for the production of Tilapia.

#### **2.4.4.2 Vegetable Waste**

Vegetable waste including trimmings, pressing fluids and rejects account for significant proportions of vegetable produce worldwide. These wastes have high content of fermentable sugars and are very perishable. As a result, they have been treated for protein enrichment by a number of processes including ensiling and solid substrate fermentation. Vegetable wastes that have been reprocessed using food grade yeasts include Chinese cabbage juice, waste brine generated from kimchi production, deproteinized leaf juices, corn silage juice, date waste, and tea process waste [203,211-215]. Stabnikova et al. [216] produced specialty selenium enriched *Saccharomyces cerevisiae* biomass by growing the organism in extracts of cabbage, watermelon, a mixture of residual biomass of green salads and tropical fruits. There are several other studies indicating that various agro-food processing wastes (substrates) have been put through various protein enrichment processes through biological conversion optimization operations employing various species of microorganisms to obtain wide range of products serving useful purposes in human food and animal feed production (Table 2.6)

### **2.5 Food Wastes As Substrates for Making Organic fertilizers**

After food wastes are degraded by aerobic microorganism, not only is the stench of the waste eliminated, but also the toxic and hazardous substances are degraded, the secondary pollution is avoided, and has good social and environmental benefits. The degradation products can be divided into organic fertilizer, bio-organic fertilizer and soil conditioner, which contain a variety of plant growth accelerating agent, and as application fertilizer can be used for flowers, trees, vegetables and others. It is a high quality organic mixed fertilizer which when applied to the cultivation of plants can improve soil structure, increase soil fertility and promote crop growth. The use of such fertilizers will not only reduce the burden of urban waste, but also promote the mass production of organic fertilizer and reduce the use of chemical fertilizers, thereby reducing the pollution of soil and water by chemical fertilizer.

Biotechnology for intensive aerobic bioconversion of sewage sludge and food waste into fertilizer has been developed (232). The wastes were treated in a closed reactor under

controlled aeration, stirring, pH, and temperature at 60 degrees C, after addition of starter bacterial culture *Bacillus thermoamylovorans*. The biodegradation of sewage sludge was studied by decrease of volatile solids (VS), content of organic carbon and autofluorescence of coenzyme F420. The best fertilizer was obtained when sewage sludge was thermally pre-treated, mixed with food waste, chalk, and artificial bulking agent. The fertilizer was a powder with moisture content of 5%. It was stable, and not toxic for the germination of plant seeds. Addition of 1.0 to 1.5% of this fertilizer to the subsoil increased the growth of different plants tested by 113 to 164%.

**Table 2.6 Some processes and organisms employed for optimization of protein enrichment of agro-food processing wastes for human food and animal feed**

Products/processes	Principal substrates	Microorganisms used	Reference
Animal feed and food; protein enriched biomass, SCP; edible mushroom; cyanogenic glycoside detoxification; Protein enriched flour; Glutamic acid; citric acid; volatile compounds	Cassava wastes (peels; slurry; bagasse; waste water); cassava tubers Cassava starch; wastewater	<i>Saccharomyces cerevisiae</i> ; <i>Lactobacillus spp</i> ; <i>Rhizopus oryzae</i> ; <i>Rhizopus spp</i> ; <i>Aspergillus niger</i> ; <i>Aspergillus spp.</i> ; <i>Cephalosporium eichhorniae</i> ; <i>Pleurotus spp</i> ; <i>Lentinus spp</i> , <i>Brevibacterium divaricatum</i> ; <i>Geotrichum fragrans</i>	[217,218] [178,181] [219] [220]
Protein enrichment; anti-nutrient removal; protein rich biomass Single cell oil; protein enriched straw/feed; single cell protein; mushroom; gamma linoleic acid; citric acid; vitamins; essential amino acids Medicinal fungus; feed	Coffee pulp; coffee husk; other coffee waste, Wheat bran/straw/corn stover/buckwheat/ millet/sugar beet pulp/citrus waste/water hyacinth; Mustard straw; bean straw; agave bagasse Agro-residues; Perennial grass	<i>Streptomyces</i> ; <i>Pleurotus spp</i> . <i>Microsphaeropsis sp</i> ; <i>Streptomyces cyaneus</i> ; various <i>Basidiomycete fungi</i> ; <i>Coprinus-fimetarius</i> <i>Micromycetes</i> ; <i>Phanerochaete chrysosporium</i> ; <i>Pleurotus ostreatus</i> ; <i>Thamnidium elegans</i> ; <i>cellulolytic bacteria</i> ; <i>Neurospora sitophila</i> ; <i>Rhodotorula gracilis</i> ; <i>Trametes spp</i> ; <i>Ganoderma spp</i> ; <i>Coriolus versicolor spp</i> , <i>Trichoderma spp</i> , <i>Lentinus edodes</i> ;	[209] [221] [222] [223] [224] [225] [226]
protein-rich fungi and feed; single cell protein;	Apple pomace; apple waste; apple pulp; grape waste; carob pod; pineapple waste;	<i>Rhizopus oligosporus</i> ; <i>Candida utilis</i> and <i>Pleurotus ostreatus</i> ; <i>Kloeckera-apiculata</i> ; <i>Penicillium funiculosum</i> <i>Myrothecium verrucaria</i> <i>Aspergillus niger</i> ; <i>Saccharomyces spp</i> ;	[227] [228] [208] [21] [143] [142] [229] [230,231] [205]
Biofuel (Biobutanol, bioethanol) as alternative energy sources	Oil palm empty fruit; Oil palm decanter cake(OPDC)	<i>Clostridium botulinicum</i>	[267] [ 270]

More than 200,000 tons of red beet are produced in Western Europe annually, most of which (90%) is consumed as vegetable. The remainder is processed into juice, colouring foodstuff and food colorant, the latter commonly known as beetroot red. Though still rich in betalains, the pomace from the juice industry accounting for 15–30% of the raw material is disposed as feed or manure.

Carrot juices and blends thereof are among the most popular non-alcoholic beverages. Steady increase in carrot juice consumption has been reported from various countries. Despite considerable improvements in processing techniques including the use of depolymerizing enzymes, mash heating, and decanter technology, a major part of valuable compounds such as carotenes, uronic acids, and neutral sugars is still retained in the pomace, which could be disposed of, usually as feed or as fertilizer.

### **2.5.1 Palm Kernel Wastes As Substrate for organic fertilizer**

Palm kernel oil (white palm oil) is obtained from the seed known as kernel or endosperm. When the oil has been extracted, the residue known as 'palm kernel cake' (PKC) is rich in carbohydrate (48 per cent) and protein (19 per cent) and is used as cattle feed [233]. The ash contains large amounts of potassium. When the PKC is further solvent extracted to remove oil, it becomes 'palm kernel de-oil cake' which has little or no nutritional value (carbon 42.73%, nitrogen 0%, volatile matter 67.71% and calorific value 4031 Kcal/Kg) and is mostly used as fuel source in industry. As PKC is deficient in nitrogen, there is need to amend it with additional nitrogen if it has to be converted into compost. In the West African communities livestock wastes are readily available and thus become a rich source for nitrogen amendment. The most common livestock which are reared around the farms and residences are goats and sheep, poultry and to some extent piggery. Cows are common in certain parts. Kolade et al. [234] developed a process of converting PKC into compost using poultry manure, and goat manure as supplements. Composting was carried out using combinations of PKC and poultry manure (3:1 ratio) and PKC and goat/sheep manure (3:1 ratio). The composting was carried out in locally made woven baskets which facilitate natural aeration of the composting material. The amount of waste in each basket was 10 Kg (7.5 Kg PKC and 2.5 Kg livestock waste) and kept in a green house at the University. A clean plastic sheet was spread under each basket to collect the leachates and putting back on to the composting material. The composting was carried out for six weeks. The composition of the raw materials and the finished product were determined.

The quality of the finished composts was assessed by following the nutrient levels, C: N ratio, moisture level and texture. The compost quality was within the acceptable limits. The compost made from PKC and goat manure however showed higher nitrogen and phosphorus levels which are needed for crop production in Nigeria. Increasing the nitrogen levels in the composts prepared from wastes is a challenge and supplementing with natural sources of nitrogen is more environmentally friendly than opting for mineral sources [235]. The results of growth experiments using the test crop *Amaranthus* spp indicate that the performances of composts prepared from PKC+poultry manure and PKC + goat manure were comparable when applied at 4 tons/Ha with those of Organo-mineral fertilizer or chemical fertilizer (NPK 15-15-15).

## **2.6 Agro-Food Wastes for Production of Biogas, Biofuels, etc. in Alternative Energy Generation**

This is a very important emerging area, particularly in the light of rapidly rising costs associated with energy supply and waste disposal and also, the increasing public concerns with regards to environmental quality degradation. Viewed from the angle that sustainability is a key principle in natural resource management, and it involves operational efficiency, minimization of environmental impact and socio-economic considerations, all of which are interdependent [236], it has become increasingly obvious that continued reliance on fossil fuel energy resources is unsustainable, owing to both depleting world reserves and the green house gas emissions associated with their use. Therefore, there are vigorous research initiatives aimed at developing alternative renewable and potentially carbon neutral solid, liquid and gaseous biofuels as alternative energy resources. Wastes from various plants can also serve as sources of energy for firing boilers in such plants. A typical example is found in the rice processing plants in which raw rice is first of all dehusked, with such husks constituting serious environmental concern since it would not be easy to dispose of them. However, such husks can be collected and used to fire boilers during the parboiling of paddy. This will save such an establishment a lot of money that could be channeled into other sectors of the plant. Another example is found in the groundnut processing plant. Dried groundnut shells are usually used as alternate energy provider during groundnut oil production. Husks, hulls, chaff and stalks generated in other cereals and pulse processing industries as well as in the spices and condiments processing plants are also used as fuel to generate heat. In recent times husks from the cereals and grains processing factories have found use in the production of briquettes, which are now used for domestic heating.

Domestic food wastes can also be sources of biogas that can be used in plants. Due to relatively high moisture content of food wastes, bioconversion technologies such as anaerobic digestion are more suitable compared to thermo-chemical conversion technologies such as combustion and gasification. Anaerobic digestion has been widely applied for treatment of organic wastes that are easily biodegradable. Zhang et al. [237] studied the characterization of food wastes as feedstock for anaerobic conversion. The anaerobic digestibility and biogas and methane yields of the food wastes were evaluated using batch anaerobic digestion tests performed at 50°C. The methane yield was determined to be 348 and 435mL/g volatile solids (VS) respectively after 10 and 28 days of digestion, while the average methane content of biogas they obtained was 73%. They concluded that the food wastes were highly desirable substrates for anaerobic digesters with regards to their high biodegradability and methane yield. Heo et al. [238] evaluated the biodegradability of a traditional Korean food consisted of boiled rice (10–15%), vegetables (65– 70%) and meat and eggs (15–20%) and reported that after 40 days a methane yield of 489mL/g VS could be obtained at 35°C. Cho and Park (212) determined the methane yields of different food wastes at 37°C and 28 days of digestion time. They were 482, 294, 277 and 472mL/g VS for cooked meat, boiled rice, fresh cabbage and mixed food wastes respectively, which corresponded to 82%, 72%, 73% and 86% of the stoichiometric methane yield respectively, based on elemental composition of raw materials. Wang et al. [239] studied the anaerobic batch digestion at 35°C of food waste using laboratory and pilot-scale hybrid solid–liquid anaerobic digesters. Their results showed that the methane contents of the biogas produced were 71% and 72% respectively. The total VS destruction for food waste after digestion was 77% and 78% after 10 and 25 days of digestion respectively. Again, since the total concentration of each of macro and micronutrients nutrients will not change significantly

during digestion of food wastes [240], the digester effluents would also provide the essential elements for plant growth, if they are used as organic fertilizers.

Much as this serves as viable option in providing alternative energy resource, there is however some contending issues needing balanced resolution. The nature and quantity of wastes generated from agriculture, food and other industries vary in types and processing technology. Similarly, the need to reuse available waste varies with the pressure on calorie and the environment. In economies that produce mostly grains, large quantities of straw are produced, while the economies that produce mainly root and tuber crops will contend with high starch-containing wastes. Although it is known that reuse of these wastes can significantly improve the economy, their effective reuse has often been constrained by the available technology. In general, the major wastes from agricultural and food industry operations include a variety of lignocellulosic materials, such as rice straw, wheat straw, maize stalk and cobs, barley straw, sugarcane bagasse, cassava bagasse, vegetable process wastes including starch wastes, sugar industry wastes as well as farm animal refuse and waste from slaughter house and fisheries operations. Food processing industries also generate large quantities of rejects, trimmings and other substandard food materials that do not make it into the production chain. Fruit processing industries produce vast quantities of wastes such as pomace and pulp, which present disposal problem; and from the fermentation industries, vast amounts of spent media and microbial biomass are also generated.

The alternate energy resources akin to first generation biofuels derived from terrestrial crops such as sugarcane, Sugar beet, maize and rapeseed place an enormous strain on world food markets, contribute to water shortages and precipitate the destruction of the world's forests. Though the second generation biofuels derived from lignocellulosic agriculture and forest residues and from non-food crop feedstocks address some of the above problems, there is the concern over competing land use or the compelling land use changes required. It is important to note however, that biomass from agricultural land must be used for the production of food and not fuel. Therefore, based on current knowledge and technology projections, third generation biofuels specifically derived from microalgae are considered to be a technically viable alternative energy resource that is devoid of the major drawbacks associated with first and second generation biofuels. Microalgae are photosynthetic microorganisms with simple growing requirements (light, sugars, CO<sub>2</sub>, N, P, and K) that can produce lipids, proteins and carbohydrates in large amounts over short periods of time. These products can be processed into both biofuels and valuable co-products in a process termed biorefinery. Biorefinery according to Fredga and Måler [241] is the sustainable processing of biomass into a spectrum of marketable products like heat, power, fuels, chemicals, food, feed, and materials.

### **2.6.1 Bio-Fuels and Biogas**

Biofuel (Biogas) technology provides an alternative source of energy to fossil fuels in many parts of the world. Using local resources such as agricultural crop remains, municipal solid wastes, market wastes and animal waste, energy (biogas), and manure are derived by anaerobic digestion [242]. These wastes are rich in lignocellulolytic materials, one of the largest and renewable sources of energy on earth. Significant improvement in biogas production occurs after pretreatment of these compounds with cellulases and cellulase-producing microorganisms. Microbial enzymatic hydrolysis of different complex organic matter converts them into fermentable structures, leading to production of biogas. Significant

improvement in biogas production occurs when crude and commercial enzymes are used in the pretreatment of complex organic matter.

Bio-fuels can be broadly classified into two major types, gaseous and liquid biofuels. Purification of the conventional biogas into methane-enriched biofuel led to the development of biomethane. Biohydrogen is a relatively new type of gaseous biofuel, which is produced by anaerobic fermentation of agricultural wastes by the synergistic action of a consortium of methanogenic, acidogenic and hydrogenic bacteria [243]. On the other hand, liquid biofuels have recently been classified into bioethanol and biodiesel. While bioethanol has recently gained rejuvenated importance in the wake of present energy crisis worldwide, biodiesel occupied the centre stage as a potential substitute for petroleum diesel in the last two decades. The conventional biogas, which is produced in biogas plants employing anaerobic digestion of organic wastes including manures by mixed microbial cultures, is composed primarily of methane (typically 55%–70% by volume) and carbon dioxide (typically 30%–45%) and may also include smaller amounts of hydrogen sulfide (typically 50–2000 ppm), water vapor (saturated), oxygen, and various trace hydrocarbons [244,245]. Due to its lower methane content (and therefore lower heating value) compared to natural gas, biogas use is generally limited to engine-generator sets and boilers [246]. Various types of the gaseous and liquid biofuels are discussed below:

### **2.7.2 Biomethane**

Biomethane is upgraded or sweetened biogas after the removal of the bulk of the carbon dioxide, water, hydrogen sulfide and other impurities from raw biogas. From a functional point of view, biomethane is extremely similar to natural gas (which contains 90% methane) except that it comes from renewable sources. [246]. Biogas can also be purified and upgraded and used as vehicle fuel. Over a million vehicles are now using biogas and fleet operators have reported savings of 40–50% in vehicle maintenance costs [247]. Anaerobic digestion has proved to be the most feasible strategy for biogas production from agricultural wastes. Lipids (characterized as oil, grease, fat, and free long chain fatty acids, LCFA) are a major organic compound in wastewater generated from the food processing industries and have been considered very difficult to convert into biogas. Improved methane yield has been reported in the literature when these lipid-rich wastewaters are pretreated with lipases and lipase-producing microorganisms. The enzymatic treatment of mixed sludge by added enzymes prior to anaerobic digestion has been shown to result in improved degradation of the sludge and an increase in methane production [242]. The potential substitution of fossil fuel with biogas represents an annual reduction in the atmosphere of 1.05 million m<sup>3</sup> of CO<sub>2</sub> [248]. Methanogenesis is methane production by methanogens via microbial decomposition of organic matter in anaerobic environments, such as river and lake beds. It is estimated that 1% of plant material formed per year by photosynthesis is remineralized via methane with an estimated 10<sup>9</sup> tons of combustible gas being produced [249]. This is methane which is naturally produced and not harvested. Instead it is oxidized, buried leading to methane deposits or more critically diffuses into the atmosphere. Atmospheric methane leads to an increase in the green house effect as it is a potent green house gas. But it is important to note the process is not carried out solely by a single microorganism but by syntrophic associations. There are a number of stages in the production of methane from agricultural residues. These stages are not always clearly defined. These stages include Hydrolysis, Acidogenesis, Acetogenesis and finally afore mentioned Methanogenesis. Typically, in a freshwater environment, plant material in the form of glucose from cellulose is completely decomposed to CO<sub>2</sub> and CH<sub>4</sub>. This is carried out primarily by the fermentation of large complex polymers, such as carbohydrates or proteins, to Carbon Dioxide (CO<sub>2</sub>), Hydrogen

(H<sub>2</sub>) acetate or formate, followed by the conversion of these substrates to methane. Methanogens have a wide range of optimum temperatures for gaseous production. But research has highlighted increased temperature not to be an advantage in production times and cost. The end product of these stages is termed *Biogas*.

The constituents of the Biogas are methane (CH<sub>4</sub>), carbon dioxide (CO<sub>2</sub>) making up approximately 90%. Other impurities such as hydrogen sulphide, nitrogen, hydrogen, methylmercaptans and oxygen complete the unrefined fuel source [250]. The refinement to almost total methane introduces the term Biomethane and increases the value in terms of energy of the end product. Processes involved can be Water Scrubbing, Pressure Swing Absorption Technologies, Chemical Absorption, Membrane Separation etc. These processes facilitate the introduction of Biomethane to the national gas grids for home use. Co-digestion of different types of organic by-products has been increasingly applied in order to improve plant profitability through easier handling of mixed wastes; common access facilities and the known effect of economy scale are some of the advantages of co-digestion. LHL (Laying Hen Litter), CW (Cheese Whey), SW (Slaughterhouse Wastewater), cattle manure, swine manure or piggery effluent have been utilized as biogas yield ameliorating agents [251]. Co-digestion of OMW (Olive Mill Wastewater) and WGR (Wine Grape Residues) with SW yields much improved results, with a 30–57% increase in methane yields as compared to individual digestion of the substrates. Methane yields during thermophilic digestion are 14–35% higher than mesophilic digestion. Thermophilic digestion, intrinsically, has higher degrading capability and methanogenic activity in biogas production. Results of comprehensive studies suggest that thermophilic anaerobic digestion may be attractive for treating high-temperature industrial effluents and specific types of slurries [247]. A simple laboratory biogas digester is shown in plate 2.1 [247]

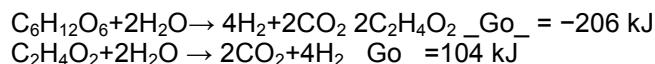


**Plate 2.1 A typical laboratory biogas digester (SORCE: Parawira [247])**

### **2.7.3 Biohydrogen**

Hydrogen is a very high energy (122 kJ/g) yielding fuel in comparison to methane or ethanol, produces water instead of greenhouse gases when combusted. Photoautotrophically

growing bacteria and micro-algae, utilize light as primary energy source to split water into hydrogen and oxygen by the enzyme hydrogenase. The basic reactions are [252]:

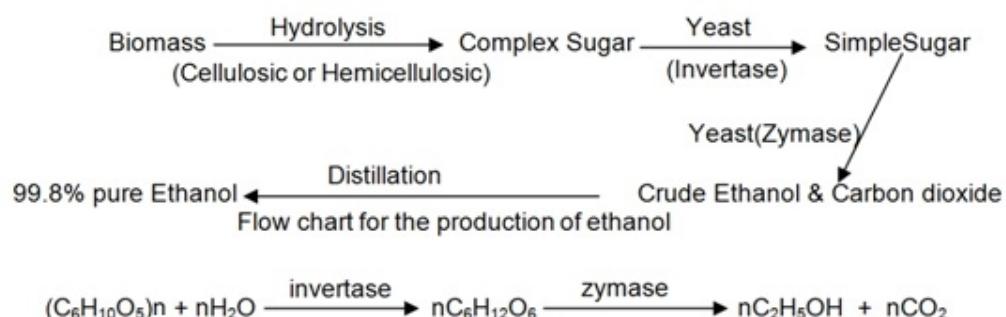


The equations show how biomass containing carbohydrates is converted into organic acids and hydrogen by the process of thermophilic heterotrophic fermentation. The organic acids are subsequently converted into hydrogen by photoheterotrophic fermentation process. During growth of thermophilic bacteria, hydrogen production is directly linked to central metabolic pathways unlike the case during photoheterotrophic growth [252].

**Production of Hydrogen from Agro-food Industrial Wastes:** Several forms of organic waste streams, ranging from solid wastes like rice straw, black strap molasses [253] to waste water from a sugar factory and a rice winery have been successfully used for hydrogen production. Most experiments have shown considerable hydrogen production with the limited number of thermophilic strains used. The utilization of potato peels by the two phase approach has been described by de Vrie and Claassen [252]. The organic acids already present in the initial substrate and additionally produced in the first fermentation step, are the substrates of choice for the photo-heterotrophic fermentation. Assessment of the total production of hydrogen and acetate from glucose and from equivalent amount of sugars in potato steam peel hydrolysate (prepared by the action of amylase and glucoamylase) revealed that higher hydrogen production occurred from the peels.

#### **2.7.4 Bioethanol**

Bioethanol is a biofuel used as a petrol substitute, produced by simple fermentation processes involving cheaper and renewable agricultural carbohydrate feedstock and yeasts as biocatalysts. A variety of common sugar feedstock including sugarcane stalks, sugar beet tubers and sweet sorghum are used. However, Lignocellulosic materials are the most abundant renewable organic resources (about 200 billion tons annually) on earth that are readily available for conversion to ethanol and other value-added products, but they have not yet been tapped for the commercial production of fuel ethanol. The lignocellulosic substrates include woody substrates such as hardwood (birch and aspen, etc.) and softwood (spruce and pine, etc.), agro residues (wheat straw, sugarcane bagasse, corn stover, etc.), dedicated energy crops (switch grass, and Miscanthus etc.), weedy materials (*Eichornia crassipes*, *Lantana camara* etc.), and municipal solid waste (food and kitchen waste, etc. )[254]. The fermentation process is mediated by two enzymes invertase and zymase, produced by the yeast cells. The overall process steps are as follows [255]:



The flow chart and equation above describes an overview of bioethanol production from cellulosic or hemicellulosic biomass. The first step is a pre-treatment step for the conversion of cellulosic and hemicellulosic biomass into complex sugars by acid catalysts or enzyme. The bioconversion of the complex sugars into bioethanol is mediated by invertase and zymase as seen above. But the ethanol produced from this fermentation process contains significant amount of water in it. To remove water, fractional distillation process is used, wherein the ethanol-water mixture is vaporized. Bioethanol gets separated from water due to lower boiling point (78.3°C). After distillation step the final product is enriched with 95% to 99.8% ethanol. The discovery of continuous mode during 1960s permits recycling of yeast; increase the speed of the process and reducing the cost. The Yield % of theoretical max in continuous process is around 95% as compared to less than 90% for batch or fed-batch [256].

Despite the success achieved in the laboratory, there are limitations to success with lignocellulosic substrates on a commercial scale. The future of lignocellulosics, according to Chandel et al. [254], is expected to lie in improvements of plant biomass, metabolic engineering of ethanol and cellulolytic enzyme-producing microorganisms, fullest exploitation of weed materials, and process integration of the individual steps involved in bioethanol production. Issues related to the chemical composition of various weedy raw substrates for biofuel (bioethanol) formation, including chemical composition-based structural hydrolysis of the substrate, need special attention. This area could be opened up further by exploring genetically modified metabolic engineering routes in weedy materials and in biocatalysts that would make the production of this biofuel more efficient. Ethanol produced from renewable and cheap agricultural products reduces the green house gas emissions like CO<sub>x</sub>, NO<sub>x</sub> and SO<sub>x</sub> and eliminate smog from the environment. Agricultural residues and wastes have several advantages as they do not require any additional lands because they are collected into piles at large agricultural and forestry facilities. Some of the residues and waste materials abundantly available that are used as potential substrates for bioethanol production in various Countries are shown in Table 2.7

**Table 2.7. Some food residues/wastes serving as major raw materials used for bioethanol production in various countries**

Residues and plant wastes	Country	Bioethanol yield (in percentage)	Reference
Switchgrass	USA	72%	[257]
Corn steep liquor (CSL)	USA and Brazil	No yield 258	
Brewer's yeast autolysate and Fish soluble waste	Thailand	88%	[258]
Waste Potato	Finland	87%	[259]
Rice straw, oat straw,wheat straw Not specified	Not specificie	-	[260]
Oil palm empty fruit, Oil palm decanter cake(OPDC)	Malaysia	Not specified	[267,270]

### **2.7.5 Biobutanol**

Biobutanol production from lignocellulosic biomass is attracting great interest due to its sustainability and superior characteristics compared to other biofuels derived from cellulosic materials. Biobutanol can be used in current petrol engines without any modifications. It

contains a high energy density less volatile content, and is less corrosive [261]. The use of agricultural biomass as fermentation feedstock could possibly reduce the biobutanol production cost which is a major problem for the biofuel industry [262]. Nowadays, butanol is chemically synthesized from petroleum-based material. However, the dependency of butanol production on petroleum is becoming an issue due to the increase in the price, and the extensive consumption of petrol [263]. It also leads to an increased carbon dioxide level in the atmosphere, which contributes to the greenhouse effect and global warming. Thus, a biological process for butanol production from biomass has been proposed. However, at present, the butanol production through acetone-butanol-ethanol (ABE) fermentation by clostridia faces several challenges. One of them is the complexity of the ABE fermentation due to biphasic conditions, in which the cells are very sensitive to certain parameters [264]. These include the initial pH which is very crucial in ABE fermentation in that it controls the metabolic shift from acidogenesis to solventogenesis. During acidogenesis, the acids (acetic and butyric) and gases (carbon dioxide and hydrogen) are produced, while solventogenesis produces solvents (acetone, butanol and ethanol) [265]. In addition, the balance of the concentration of sugars is important to prevent substrate inhibitions that subsequently reduce the biobutanol yield. A very low sugars concentration might reduce the cells growth and interrupt the acidogenesis phase, thus inhibiting the formation of solvents [266]. Additionally, ABE fermentation requires a sufficient amount of nitrogen for generating new bacterial cells, where then the carbon to nitrogen ratio becomes an important parameter [267,268]. The imbalance value of any of the parameters will inhibit the cells metabolism, thus reducing the production of solvents. On account of all these, a multifactorial optimization for increase in biobutanol production has been proposed [269,267,270].

Fortunately for us in Nigeria, we have in great abundance a ready source of one such lignocellulosic waste material that could be harnessed for biobutanol production. Oil palm trees are in great abundance here, particularly in the Southern part of the country where a sizable percentage of the populace engage in processing of palm fronts into palm oil, with oil palm decanter cake(OPDC) as one of the waste products of the oil palm industry. In an optimized reaction conditions as proposed [267,269,270], a good amount of biobutanol fuels are produced through the fermentation of OPDC hydrolysate sugars using appropriate organisms such as *Clostridium acetobutylicum* ATCC 824.

#### **2.7.5.1 Lignocellulosic materials**

Removal of lignin from lignocellulosic raw materials is the most critical step. Among various methods, physicochemical and biological methods are mainly used for pre-treatment. Saturated steam at 160°C to 290°C and at high pressure (0.69 to 4.65MPa) is used to convert hemicelluloses into soluble oligomers [271,272]. Lignin is not solubilized but redistributed. Ammonia soaking of corn stover at room temperature can remove as much as 74% of the lignin [257]. The fungus *Phanerochaete chrysosporium* can also be used for degrading lignin [256].

#### **2.7.5.2 Non-lignocellulosic materials thippi**

Non-lignocellulosic Materials Thippi is an agro-industrial waste composed of starch, pectin, fiber and protein. After pre-treatment at 121°C for 20 minutes, acid or enzymatic treatment is done. Acid treatment is done with 0.75% H<sub>2</sub>SO<sub>4</sub> at 55°C for 3h. Enzymatic treatment is performed with various enzymes like amylase, pectinase and cellulase at 55°C for 3 h at pH 5. Fermentation is carried out to use the reducing sugars obtained from above process to produce ethanol [273]. Because of higher yield in percentage of maximum theoretical value

(>90%) as compared to other available non-lignocellulosic materials, thippi can be used as preferred substrate with great potential for bioethanol production.

Switchgrass (*Panicum virgatum*): is a perennial grass grown in warm season and resistant to harsh conditions, pests and diseases. It is capable of producing high biomass yields at low fertilizer application rates. Untreated switch grass contains 42% cellulose, 31% hemicellulose, 6% acid detergent lignin (ADL), 22% kason lignin and 0.7% ash. The yield % of theoretical max is 72% with simultaneous saccharification and fermentation [257].

### **2.7.6 Biodiesel**

Finite resources and gradually increasing demand for diesel all over the world lead researchers to find some alternative sources. Emission of toxic green house gases from the combustion of petroleum diesel is also a major contributing factor for this. Right from the experiment of Rudolf Diesel using peanut oil in his self-designed engine (World Exhibition in Paris in 1900), numerous studies have followed to establish the potential of triglycerides as alternative sources of diesel. But using triglycerides directly into a diesel engine leads to some operational difficulties due to its high viscosity and poor low temperature properties like pour point and cloud point [274]. This problem could be fixed by developing vegetable oil derivatives that resemble the properties of petrodiesel. Transesterification is the most widely used process in which triglycerides react with an alcohol (mainly methanol) in the presence of a chemical (acid or alkali) or biological (enzyme) catalysts to produce mono alkyl esters, popularly known as biodiesel. Some alkali- catalyzed batch processes have been commercialized.

Rapidly spiraling crude oil prices and cost ineffectiveness of most of the biofuel technologies, mainly due to expensive raw materials and manufacturing processes, have fueled extensive worldwide search and utilization of agro-industrial residues for the cost competitive production of alternative biofuels. The major gaseous biofuels, namely, biomethane and biohydrogen and the major liquid biofuels, namely, bioethanol and biodiesel have evolved as potential alternative to the dwindling fossil fuel resources. Bioethanol and biodiesel are gaining importance as alternative fuels to petrol and diesel respectively. Bioethanol, which is conventionally produced from cane molasses by yeast fermentation, can also be produced from various residues and wastes. Efficient process optimization and integration by combining production and recovery processes may lead to economic production of bioethanol. Switchgrass that grows mainly in the USA in drastic climatic conditions and contains high percentage of cellulose and hemicelluloses generated some excitement in the field of bioethanol production. Biodiesel on the other hand is generally produced from vegetable oils.

#### *2.7.6.1 Production of biodiesel from agricultural wastes*

Biodiesel is generally produced from vegetable oils or animal fats. Various oils like palm oil, soybean oil, sunflower oil, rice bran oil, rapeseed oil etc. are used. The choice of vegetable oil used depends on its abundant availability in the country where biodiesel is produced. However, bioethanol produced from wastes can in turn be used for the transesterification of vegetable oils to produce mono ethyl esters of fatty acids as biodiesel. Some residues can be successfully utilized as carbon sources for single cell oil production. It reduces the fermentation costs [275]. Whey concentrate and tomato waste hydrolysate, which contains more than 1 g/l total organic nitrogen, in turn produce 14.3 % and 39.6 % lipid respectively can be used for gamma-linolenic acid production. The amount of gamma-linolenic acid

produced from these wastes is 14.1% and 11.5%, which makes whey concentrate and tomato waste hydrolysate, two good raw materials for biodiesel production [276].

Biotechnological techniques to produce biofuels from agro-industrial wastes and residues are potentially effective in reducing the emission of toxic pollutants and greenhouse gases, saving our environment and partly solving the worldwide fuel crisis. By focusing the transformative power of biotechnology on challenges in biofuel production while considering sustainability in all its dimensions, one can reasonably hope to enable the 'second industrial revolution' that our society now requires. In order to make rational and balanced judgment so vitally required in this matter concerning the sustainability of energy supply chains and energy systems as important factors for consideration, there is need for Life cycle analyses (LCA), which provides the conceptual framework for a comprehensive comparative evaluation of energy supply options with regard to their resource requirements as well as the health and environmental impact. Full scope LCA of a production plant for instance, considers not only the emissions from plant construction, operation and decommissioning, but also the environmental burdens and resource requirements associated with the entire lifetime of all relevant upstream and downstream processes within the energy chain.

## **2.8 Wastes As Substrate for Producing of Food additives**

Pectin is a gelling agent. It creates bonds with water (pectin-water) and with itself (pectin-pectin). Pectin-pectin bond gives the gel the strength and pectin-water bond gives jelly its softness. A different proportion between those two types of bonds gives jam or jelly a different texture. Pectin is one of the most versatile stabilizers available. Pectin's gelling, thickening and stabilizing attributes makes it an essential additive not only in jams and jellies but also in the production of many other food products, as well as in pharmaceutical and medical applications. The citrus industry generates a lot of peels during the production of various types of citrus fruit juices. Such peels are now used for the production of pectins [44] that are used as thickeners in such products as jams. A new method for the production of pectin from citrus peel was developed by Sakai and Okushima [277]. For this purpose, a microorganism which produces a protopectin-solubilizing enzyme was isolated and identified as a variety of *Trichosporon penicillatum*. This was used in the production. The most suitable conditions for the pectin production were determined as follows. Citrus (*Citrus unshiu*) peel was suspended in water (1:2, wt/vol), the organism was added, and fermentation proceeded over 15 to 20 h at 30°C. During the fermentation, the pectin in the peel was extracted almost completely without macerating the peel. By this method, 20 to 25 g of pectin was obtained per kg of peel. The pectin obtained was special in that it contained neutral sugar at high levels, which was determined to have a molecular weight suitable for practical applications [277].

Production of pectin is considered the most reasonable way of utilizing apple pomace both from an economical and from an ecological point of view. In comparison to citrus pectins, apple pectins are characterized by superior gelling properties. However, the slightly brown hue of apple pectins caused by enzymatic browning may lead to limitations with respect to their use in very light-colored foods. Apple pomace has been shown to be a good source of polyphenols, which are predominantly localized in the peels and are extracted into the juice to a minor extent. Major compounds isolated and identified include catechins, hydroxycinnamates, phloretin glycosides and quercetin glycosides. Since some phenolic constituents have been demonstrated to exhibit strong antioxidant activity *in vitro*, commercial exploitation of apple pomace for the recovery of these compounds seems

promising. Inhibitory effects of apple polyphenols and related compounds on carcinogenicity of streptococci suggest their possible application in dentifrices.

Apart from oranges, grapes are the world's largest fruit crop with more than 60 million tons produced annually. About 80% of the total crop is used in wine making, and pomace represents approximately 20% of the weight of grapes processed. From these data it can be calculated that grape pomace amounts to more than 9 million tons per year. Its composition varies considerably, depending on grape variety and technology of wine making. A great range of products such as ethanol, tartrates, citric acid, grape seed oil, hydrocolloids, and dietary fiber are recovered from grape pomace. Anthocyanins, catechins, flavonol glycosides, phenolic acids and alcohols and stilbenes are the principal phenolic constituents of grape pomace. Anthocyanins have been considered the most valuable components, and methods for their extraction have been reported. Catechin, epicatechin, epicatechin gallate and epigallocatechin were the major constitutive units of grape skin tannins. Since grape and red wine phenolics have been demonstrated to inhibit the oxidation of human low-density lipoproteins (LDL), a large number of investigations on the recovery of phenolic compounds from grape pomace have been initiated.

Mango is one of the most important tropical fruits. Mango and mango products have experienced worldwide popularity and have gained increasing relevance also in the European market. The major wastes of mango processing are peels and stones, amounting to 35–60% of the total fruit weight. Mango seed kernel fat is a promising source of edible oil and has attracted attention since its fatty acid and triglyceride profile is similar to that of cocoa butter. Therefore, legislation has recently allowed mango seed kernel fat to be used as a cocoa butter equivalent. Mango seed kernels may also be used as a source of natural antioxidants. The antioxidant principles were characterized as phenolic compounds and phospholipids. A standardized method for the recovery of good quality mango peel pectin with a degree of esterification of about 75% is now available. Mango peels are also reported to be a good source of dietary fiber containing high amounts of extractable polyphenolics. Guava is a rich source of relatively low methoxylated pectins (50%), amounting to more than 10% the dry weight. Since wastes constitute only 10–15% of the fruit, the use of guava for pectin production is limited. The seeds, usually discarded during processing of juice and pulp contain about 5–13% oil rich in essential fatty acids. The waste resulting from passion fruit processing consists of more than 75% of the raw material. The rind constitutes 90% of the waste and is a source of pectin (20% of the dry weight). Passion fruit seed oil is rich in linoleic acid (65%).

## **2.9 Wastes as Substrates for the Production of Aroma Compounds & Other Important Industrial Chemicals**

An aroma compound, also known as odorant, aroma, fragrance or flavor, is a chemical compound that has a smell or odor. Aroma compounds can be found in food, wine, spices, perfumes, fragrance oils, and essential oils. The world of aroma is very attractive especially because it concerns the taste of what we eat [278]. Aroma compounds can be extracted from fruits or vegetables but as they are required in the product in concentrations comparable to those in the source material, and this utilizes high amounts of materials and is generally not economically realistic. Most of them can also be synthesized in a chemical way, resulting in chemical compounds that are not well perceived by consumers whose demand is the flavour of natural products. As an alternative, biotechnology proposes to use enzymes or whole cells to produce aroma compounds. Flavours and fragrances constitute a

world-wide market of US\$ 7 billion a year, with a share of 25% of the food additives market [279]. The consumer's preference for natural food additives is more important than ever. Aroma compounds have been of high importance for medicine, food, perfumery and cosmetics since ancient times. Especially because of their high biological activity and low toxicity, aroma compounds are often used in pharmaceutical products. It is also used as defoaming agents for ophthalmic solutions with high concentrations of surfactants. Natural aroma compounds are used to improve the shelf life and safety of minimally processed fruits [280,281]. The renaissance of the use of natural products in the recent past also led to an increasing interest in aroma components. Although their usage in the field of traditional and classical medicine has been a subject of discussion between researchers, the number of scientific papers including analytical and biological data on aroma components is at present higher than ever. Additionally, the flavouring and conservation of food stuff by odorous volatiles, as well as the search for pleasant smelling raw materials for perfume and cosmetic products in their true nature is not only supported by the food, but also the perfume and cosmetic industry, with great commercial significance. Hence, there is need for a cheaper and readily available raw material production base for this important class of commercial products.

Some residues like coffee pulp and husk, cassava bagasse, sugar cane bagasse are generated in large amounts during processing, and their disposal rather causes serious environmental problems. In recent years, there has been constant increase in the efforts to utilize these residues as substrates (carbon source) in bioprocessing [282,283], with microorganisms playing an important role in the generation of natural compounds, particularly in the field of food aromas [284,285]. Numerous microorganisms are capable of synthesizing potentially valuable aroma compounds and enzymes used in flavour manufacturing. However, yields are often disappointingly low, which hampers extensive industrial application. In the last decades there has been an increasing trend towards the utilization of the solid-state fermentation (SSF) technique to produce several bulk chemicals and enzymes [286,85,287,288]. Hence, Solid state fermentation (SSF) has been used for the production of aroma compounds by cultivating yeasts and fungi. SSF has been known from ancient times (approximately 2600 BC), and typical examples of this technique are traditional fermentations such as Japanese koji, Indonesian tempeh and French blue cheese. In recent years, SSF has received more and more interest from researchers, since several studies on colourants [289], flavours [290], enzymes [282,291], and other substances of interest to the food industry have shown that SSF may lead to higher yields or better product characteristics than submerged fermentation (SmF). In addition, costs are much lower due to the efficient utilization and value-addition of wastes [39]. The main drawback of this type of cultivation concerns the scaling up of the process, largely due to heat transfer and culture homogeneity problems [292,293]. However, research attention has been directed towards the development of designs such as rotating drum bioreactor [294], immersion bioreactor [295], and mixed solid-state bioreactor [296], which overcome these difficulties.

Several researchers have studied SSF production of aroma compounds by several microorganisms such as *Neurospora* sp., *Zygosaccharomyces rouxii* and *Aspergillus* sp., using pre-gelatinized rice, miso and cellulose fibres, respectively [297-300]. Bramorski et al. [301] compared fruity aroma production by *Ceratocystis fimbriat* in solid-state cultures using several wastes (cassava bagasse, apple pomace, amaranth and soybean), and found that the medium with cassava bagasse, apple pomace or soybean produced a strong fruity aroma. Soares et al. [302] also reported the production of strong pineapple aroma when SSF was carried out using coffee husk as a substrate by this strain. Other important industrial

chemical compounds such as acetaldehyde, ethanol, ethyl acetate (the major compound produced), ethyl isobutyrate, isobutyl acetate, isoamyl acetate and ethyl-3-hexanoate were identified in the headspace of the cultures. The addition of leucine increased ethyl acetate and isoamyl acetate production, and then a strong odour of banana was detected. Bramorski et al. [299] and Christen et al., [303] described the production of volatile compounds such as acetaldehyde and 3-methylbutanol by the edible fungus *Rhizopus oryzae* during SSF on tropical agricultural substrates. The production of 6-pentyl-a-pyrone (6-PP), an unsaturated lactone with a strong coconut-like aroma, was studied using liquid and solid substrates by De Araujo et al. [304]. While Sugarcane bagasse was adequate for growth and aroma production, it has been demonstrated that, by solid-state fermentation process, it is possible to produce 6-PP at higher concentration than that reported in literature for submerged process. *Kluyveromyces marxianus* produced fruity aroma compounds in SSF using cassava bagasse or giant palm bran (*Opuntia ficuindica*) as a substrate [305]. Solid Substrate Fermentation was found to be very suitable for the production of pyrazines. Besson et al. [298] and Larroche and Besson [306] studied the biosynthesis of 2,5-dimethylpyrazine (2,5-DMP) and tetramethylpyrazine (TMP) using SSF cultures of *Bacillus subtilis* on soybeans. Production of dairy flavour compounds, such as butyric acid and lactic acid in mixed cultures of *Lactobacillus acidophilus* and *Pediococcus pentosaceus* growing on a semisolid maize-based culture has been reported [307]. Soccol et al. [308] studied the synthesis of lactic acid by *Rhizopus oryzae* in SSF with sugarcane bagasse as a support. They obtained a slightly higher productivity than in submerged cultivation. Moreover, lactic acid production by lactic acid bacteria *Lactobacillus paracasei* and *Lactobacillus amylophilus* GV6 under SSF conditions using sweet sorghum and wheat bran as both support and substrate respectively, have been investigated [309,310]. It is known that several methyl ketones such as 2-undecanone, 2-nonenone and 2-heptanone are produced at commercial scale by SSF from *Aspergillus niger* using coconut fat as substrate with a yield of 40 % [311]. Several methods have been developed in order to enable vanillin and furanone or pyranone derivatives of natural origin to be produced from agricultural wastes.

As a case study of agricultural waste transformation, Ezejiofor et al. [42] in one of their waste to wealth series, explored the industrial raw material potential of peels of Nigerian sweet orange (*Citrus sinensis*), usually available in great abundance in parts of Nigeria and other countries of the world. Citrus, a major plant source of essential oils is one of the most important commercial fruit crops grown in all continents of the world [312]. In China alone, according to the FAO report [313], citrus covers a cultivating area of 17.1 million hm<sup>2</sup>, with a production capacity of almost 16 million tons. Natural and cultivated hybrids of citrus include commercially important fruits such as the oranges, grape fruit, lemons, some limes and some tangerines. Sweet orange (*C. sinensis*) is one of the native citrus cultivars grown in China, constituting about 60% of total citrus yields and keeping a stable development [314]. In current citrus industry, emphasis are laid only on orange fruits harnessed and marketed fresh or as processed (and canned) juice, while fruit peels produced in great quantities during the process are mainly discarded as waste. For this reason, researchers have focused on the utilization of citrus products and by-products [315,316]. Thus, the peels of sweet orange are not only left out as waste but also considered as one of the major factors that hamper the development of citrus industry. In Florida alone, citrus processing yields about 1.2 million tonnes of dry orange waste most of which is currently marketed as low value feed for cattle. Of the 36.0 million tonnes world production, Nigeria produces 0.3 million tonness and has the potential to produce more orange wastes in high proportion. Though Nigeria is not well noted for the exportation of citrus fruits, she has the potential to produce more for both local and international markets. Presently, the local processing of citrus fruits is on the increase to meet increasing local demands for fruit juice that was

previously met by large-scale importation. Of all the citrus fruits, sweet orange is the commonest and the most widely cultivated and consumed in the major 15 citrus growing states in Nigeria [317]. This also means quantum increases in the volume of wastes derived from its fruit juice production. However, evaluation of industrial raw materials potential of Nigerian sweet orange peels via application of some physical and chemical characterization procedures on the extract of these peels confirmed limonene (one of the terpenes) as a dominant component of the orange peel, among others present in relatively lesser amounts [42]. Limonene is an essential oil with wide applications in industrial and domestic domains. Given the array of chemical contents of orange peel, it will amount to huge economic waste to consider the orange peels as waste garbage meant only for the waste bin. Exploring and exploiting the abundant essential oil, seemed to be an additional way to evaluate the underlying economic values of citrus due to their usefulness as food nutrient, and the special roles they play in food, flavor and cosmetics industries. Thus, extraction, identification and separation of the components of sweet orange peel is another way of enhancing the economic value and industrial application of this cultivar, providing a cheaper and safer alternative source of raw material for industrial and other useful purposes, as well as providing a safer and cheaper means of waste management through transformation of orange peel wastes to a resource for industrial wealth. Orange peel waste is a source of essential oil, a vital resource, very useful in foods, cosmetics, and pharmaceuticals, other industrial and domestic sectors of the economy.

## **2.10 Agro-Food Industrial Residues/Wastes as Substrates for Production of Secondary Metabolites: Antibiotics, Steroids, Alkaloids, etc**

Despite the obvious problems that agricultural waste can create, the vast quantities of wastes that are generated as a result of diverse agricultural and industrial practices represent one of the most energy-rich resources on the planet. Accumulation of this biomass in large quantities every year results not only in the deterioration of the environment, but also in the loss of potentially valuable material which can be processed to yield a number of value added products, such as food, fuel, feed and a variety of chemicals. The residues are generated globally, with a major portion left unutilized, and as wastes in the surrounding environment. Such wastes produced annually can be used as a natural bioresource for the production of bioactive compounds such as secondary metabolites from various selected microorganisms. Secondary metabolites are excreted by microbial cultures at the end of primary growth and during the stationary phase of growth. Secondary metabolites represent some of the most economically important industrial products and are of huge interest. The best known and most extensively studied secondary metabolites are the antibiotics, steroids and alkaloids [318].

Bioactive compounds are mostly secondary metabolites produced by microorganisms in an active culture cultivation process. Secondary metabolites usually accumulate during the later stage of microbial growth, in process of fermentation known as the "Idiophase". This later stage of microbial growth follows the active growth phase called "Trophophase". Compounds produced in the idiophase have no direct relationship to the synthesis of cell material and normal growth of the microorganisms. Secondary metabolites are formed in a fermentation medium after the microbial growth is completed. Filamentous fungi synthesize many secondary metabolites and are rich in genes encoding proteins involved in their biosynthesis. Genes from the same pathway are often clustered and co-expressed in particular conditions [319].

### **2.10.1 Agro-food wastes as substrates for production of Oxytetracycline and other antibiotics**

Oxytetracycline, a broad-spectrum antibiotic, is a bacteriostatic antibiotic that inhibit protein synthesis by binding reversibly to the 30S ribosomal subunit of the microorganism. It is therefore a very important class of antibiotics, and is used in human and veterinary medicine and as a supplement in poultry and swine production, preservation of fish, meat and poultry [320,321]. It is also used in non-therapeutics for the control of plant diseases, stimulation of amino acid fermentation and inhibition of material biodeterioration [322,323]. Oxytetracycline and other antibiotics had been produced by variety of methods. Some may be obtained from a semi solid culture where low water content and high degree of aeration at the surface favours the production of antibiotics [320]. Oxytetracyclines had been produced from a widerange of organic compounds by various strains of *streptomyces* organism predominantly found in the soil and decaying vegetation. Several food processing wastes and by-products such as sweet potato residue [324], saw dust, rice hulls and corn cob [320], cassava peel, corn pomace, corncob, and groundnut shell [323] and cocoyam peels [43] have all served as effective substrates for the production of antibiotics by solid-state fermentation. Solid-state fermentation is defined as the process in which microbial growth and products formation occur on the surfaces of solid substrates in the near or absence of water. *Streptomyces*, the largest genus of actinobacteria are a group of Gram-positive bacteria that generally have high guanine-cytosine (GC) content [325,326]. They are predominantly found in the soil, decaying vegetation and noted for their distinct earthly odour which result from the production of a volatile metabolite geosmin [327]. They make use of wide range of organic compounds as sole sources of carbon for energy and growth [328]. The optimum temperature is 25 to 35°C; some species grow in temperatures within the psychrophilic and thermophilic ranges. The optimum pH range for growth is 6.5 to 8.8. They have over 500 species including *Streptomyces ambofaciens*, *Streptomyces noursei*, *Streptomyces griseus*, *Streptomyces hygroscopicus*, *Streptomyces rimosus*, *Streptomyces speibonae*, etc [327], with a few species being pathogenic for animals, plants and humans. Their ability to produce oxytetracycline from a widerange of organic compounds has been quite phenomenal. Equally phenomenal is the wider applications/ consumption of this antibiotic because of its broad spectrum nature. This means that for a long time to come there would continue to be a need for this drug. Therefore, the search for alternative substrates for enhanced production base of oxytetracycline would possibly remain an endless one for a long time to come [43]. Hence, the further processing of agricultural or food wastes from many organic compounds such as corncob, groundnut shell, cassava peel, corn pomace [323]; Saw dust, rice husks and corn cobs [320] and cocoyam peels [43] as substrates for oxytetracycline production are indeed some positive responses to the call for enhancement of production base of this very important antibiotic, and such applications have far reaching implications for environment, safety and health, and ultimately for the economy. For instance, Cocoyams (the third most important tuber crop cultivated after yam and cassava), belonging to the genera *coccosia* and *xanthosoma* and family Araceae, are tropical flowering plant grown primarily for the starchy corms. Cocoyams have over the years been a permanent feature of the farming system in South East and South West, Nigeria. The starchy corms serves as a dietary fibre, which can be boiled, consumed with palm oil or used in making sauce or fried and served as chips using the species *X. esculenta*. In all these instances or applications, their peels remain wastes which must be disposed off immediately or it decomposes, becoming nuisance and a source of environmental degradation/pollution [43]. By so converting these peels, their nuisance values, environmental menace potentials and their consequent health implications have been drastically reduced, if not totally

eliminated, while turning them into resource for industrial growth, wealth creation and economic prosperity. Plates 2.2-2.4 below exemplify typical steps of transformation of waste (cocoyam peels or residue) through fermentation to Oxytetracycline, an important antibiotic, thus impacting positively on the environment, pharmaceutical industry, health, economy, and therefore wealth of the populace.



**Plate 2.2. Residue of two species of Cocoyam (*Xanthosoma esculenta* and *Colocasia esculenta* (Substrates for fermentation) in the Production of Oxytetracycline by Ezejiofor et al. [43]**



**Plate 2.3. Fermenting substrates for production of Oxytetracycline by Ezejiofor et al. [43]**



**Plate 2.4. Extracted Oxytetracycline in a sterile sample bottle by Ezejiofor et al. [43]**

#### **2.10.2 Agro-food wastes as substrates for fungal production of mycotoxins, secondary metabolites**

A variety of agricultural residues such as wheat straw, rice hulls, spent cereal grains, various brans such as wheat and rice bran, and corncobs, are available globally, which can be considered the cheaper and often free of cost substrates for the commercial production of secondary metabolites [329]. The following points are worth consideration for the application and suitability of solid agricultural wastes in the biosynthesis of fungal secondary metabolites [319]:

1. In several productions, the product formation has been found superior using solid insoluble substrates.
2. The most commonly used microorganisms in the production of secondary metabolites are fungi and *Actinomycetes*; and the mycelial morphology of such organisms is ideal for their invasive growth on solid and insoluble substrates.
3. The fungal morphology is responsible for considerable difficulties in largescale submerged processes. These include highly viscous, non-Newtonian broths and foam production. This results in very high power requirements for mixing and oxygen transfer. The presence of chemical antifoam in fermentation broths reduces oxygen transfer efficiency and can lead to problems in the product recovery.
4. In some processes, the final product is required in form of solid consistency, such as antibiotics present in animal feed.
5. The capital cost of overall production process using solid substrates is claimed to be significantly less.
6. The yields of certain secondary metabolites such as aflatoxin B1 and ochratoxin A obtained from liquid culture were found to be very poor. This led to the use of solid substrates and subsequently, a higher yield of 100 g. Similarly the production of the

cyclic pentapeptide mycotoxin, malformin C was performed using *Aspergillus niger* in solid culture and a higher yield of 369 mg/kg was obtained compared to the yield of 15–200 mg/kg from liquid fermentations [330]. The production of extremely toxic mycotoxins by fungi has attracted attention, due to their importance in human and animal food chain. The aflatoxins have considerable economic impact; the poultry industry in the U.S. lost US\$100 million per year from aflotoxin poisoning in the 1970s. Solid state cultivation has been used to produce sufficient quantities of these compounds for toxicity studies and these cultivations have been performed to study the conditions that promote toxin formation on cereal grains [331]. The production of gibberellic acid in SSF has been adopted to eliminate the need of cell-removal in downstream-processing after submerged culture process, which contributes a significant part in the production cost.

## **2.11 Wastes as Substrates for Paper Making**

Cereal straw is one of the most abundant, annually renewable resources in the world. According to a valid data, there are as many as 2.9 billion tons of cereal straw produced per year all over the world, and only in China there is 0.7 billion tons cereal straw produced per year [332]. However, such abundant resource has not attracted enough attentions and thus has not been utilized reasonably. In fact, cereal straw is a product of plants' photosynthesis, which is constituted by high percentage of macromolecule or compounds such as cellulose, hemi-cellulose and lignin. Both cellulose and hemi-cellulose are polymers made up of fermentable sugar which can be fermented into chemical materials and liquid fuel such as ethanol, acetone, acetic acid, as well as be used as the fermenting materials of antibiotics, organic acid and enzyme after hydrolysis [333]. Lignin, comprised of phenylpropane derivatives, can be further transformed to other chemicals used as the raw material in organic chemistry industry [332].

The utilization history of lignocellulose and fiber material has much to do with paper making industry which can date back to 3rd Century BC [333]. Plant fiber is the raw material in the pulp and paper industry. Nowadays, wood fiber accounts for more than 90% of the world paper; nevertheless, to those countries which lack in wood fiber, fiber material such as straw is a good substitute. China is the largest straw pulp -producing country in the world, providing more than 75% of the world's non-wood pulp [334]. Pulp and paper industry all focus on the utilization of the cellulose component in the fiber material and removal of both the hemi-cellulose and lignin components which accounts for the formation of black liquor. This process not only wastes the hemi-cellulose and lignin components, but the removal step dramatically generates the increment of the cost, and the black liquor pollutes the nearby environment especially the water resources. Obviously, it is urgent to develop new technology for straw utilization in solving the problems mentioned above. Fortunately, there are researchers who bring out biotechnologies such as bio-pulping [335] bio-bleaching and enzymatic deinking [332] to tackle the pollution problem.

## **3. CONCLUSION**

Indeed, generation of wastes remains major fallout of almost every activity of man including agro-food production and processing activities, and these has far reaching implications for environment and health generally, demanding that measures must be taken to manage them if the unpleasant consequences of their accumulation must be averted. Unfortunately, most waste management options adopted over time had tended to see waste as useless entity

that must be disposed off wholesomely. However, a close look at most natural systems gives a verdict that omnipotent and omniscient creator abhors wastages of any kind, since nature has a way of recycling every bit of its resources into usefulness. Keying into this, biotechnological concepts see wastes from a different perspective, since what is regarded as waste may not be truly totally so. This is particularly evident in agro-food wastes because most of what is often considered as wastes consigned to the waste bins contain reasonable percentage of salvageable (biomass) resources that are bioconvertible into useful products entrapped therein. With respect to these, the role of modern technology, particularly biotechnology in resource exploitation can hardly be overstated, as is evident in the wide range of useful resources: animal foods and feeds, biofertilizers, industrial chemicals/raw materials, biofuels, biogas and other energy renewable alternatives, etc. derived out of the so-called waste, using the tool of biotechnology. Thus, based on the enormous resources recoverable from agro-food processing wastes using the tool of biotechnology, this paper has convincingly and conclusively shown that wastes are veritable resources for wealth creation, and economic prosperity, since these products serve various purposes ranging from being consumer products in themselves or raw materials for production of various other products with various optional uses or applicability in different sectors of the economy.

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## **COMPETING INTERESTS**

The authors hereby declare that no competing interests exist.

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