



**VALORIZATION OF POTENTIAL ORGANIC SOLID WASTE
INTO ENZYMES/BIOCATALYST FOR SUSTAINABLE INDUSTRIAL
APPLICATIONS**

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ABSTRACT

One of the greatest obstacles in today's world is waste management. Millions of tonnes of organic solid wastes are generated every year. Organic solid wastes are a rich source of polysaccharide components. Converting these waste products into value-added products is required today. The polysaccharides present in the waste act as microbial nutrients, allowing microbial biotreatment of organic waste to produce industrially important enzymes. Solid and submerged fermentation is the technology that looks most promising for producing enzymes on an industrial scale from organic solid waste. SSF provides the natural environment to the microorganisms and better conditions for microorganism growth and enzyme production than submerged environments. Several microorganisms, including bacteria, fungus, and yeast, utilise waste as a source of energy, and enzymes are produced under suitable conditions via software. This study gives an extensive summary of organic solid waste as a raw source for synthesizing commercial enzymes. In addition, a detailed analysis was carried out on the production techniques and optimum conditions for its specific activity and their applications using various organic solid waste was provided.

Keywords: Industrial Enzymes, Solid waste, Fermentation, Microorganism.

1. INTRODUCTION

In recent years, the amount of organic waste released into the environment has increased at an alarming rate. Due to urbanization and the improvement of living standards, there was an increase in urban solid organic waste worldwide, and organic food waste was an important

part of it. According to Ariunbaatar et al., 2014, by 2025, food waste generation will increase to 44%, and organic solid waste (OSW) treatment will become a major global problem. The world's interest in producing biochemical products (organic acids, enzymes, biopolymers and so on) from organic waste is growing by the day (Blanco et al., 2016). Enzymes are water-soluble biocatalytic proteins and are widely used in industrial processes. In the past decade, the use of enzymes has increased dramatically, especially in the fields of biofuels, food improvement, laundry, biomedical research, pharmaceuticals, and agricultural. Biocatalysts are extensively applied in several industrial uses due to their high substrate selectivity and degradability, along with their moderate and ecologically acceptable circumstances. Thus, the Enzyme synthesis is one of the foremost applications in various sectors. (Fig.1)

The most promising technology to produce enzymes using organic solid waste in industrial scale are solid state and submerged fermentation. Submerged fermentation, on the other hand, is recommended for the production of lipase, protease, and fungus species, resulting in higher yield. Due to its lower capital and energy requirements along with a simpler fermentation medium, SSF has numerous merits over submerged fermentation (SMF). The production of different enzymes from Food Waste (FW) using submerged and solid fermentation systems has been reported in various literatures. Cellulase, lipase, pectinase, lipase and amylase was produced using different types of FW via for solid-state fermentation (SSF). SSF provides an environment similar to microorganisms' natural environment and improves conditions for microorganism growth and enzyme production (Thomas et al., 2013).

The final step in the production of enzyme is the purification process. The synthesized enzymes contain many other unnecessary components, to maintain the maximum specific activity and restore the original activity as much as possible. In addition, various methods can be used to purify the enzyme. The enzymatic process involves selecting the most appropriate processing steps. The degree of enzyme purification depends on the purpose of the enzyme. There are several commercially available methods for purification and isolation of commercially produced enzymes (Prasad and Roy, 2018)

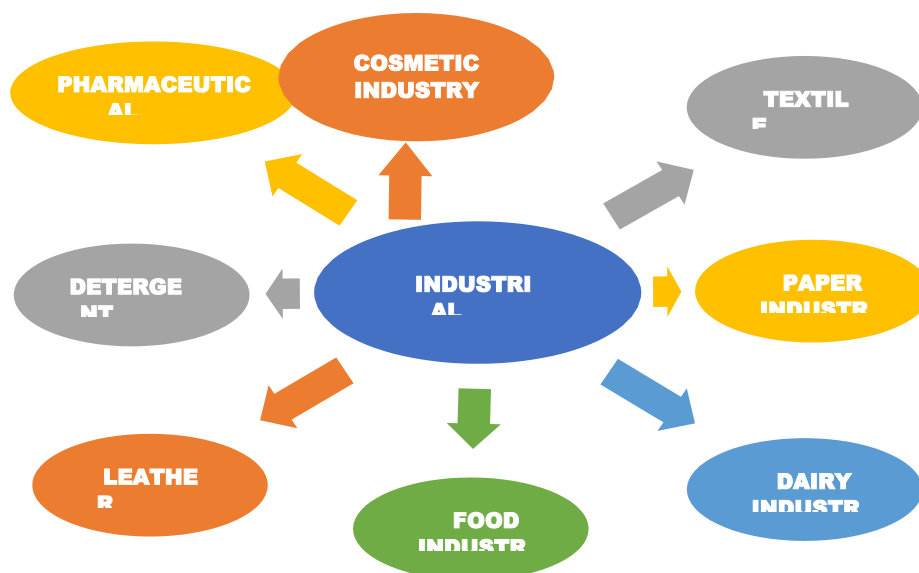


Fig. 1. Application of industrial enzymes in various industries

2. SCENARIO OF POTENTIAL ORGANIC SOLID WASTES GENERATION

Each year, the amount of organic solid wastes generated increases rapidly. Difficulties with organic waste management have become more prominent in recent years as a consequence of the world's largest rapid progress towards industrialization. The major source of organic waste is composed of agricultural residues, residential food waste, human as well as animal waste, and some other components typically employed as animal feed. As stated in this study, the utilization of OSW in various biotechnological processes and the development of considerable value added bio-items appears to be incredibly engaging and promising. OSW aids in solid waste management and pollution reduction while also cutting operating and manufacturing expenses. A wide range of organic solid waste may be easily exploited and converted into important bioproducts such as bioethanol, bioethanol, enzymes, biosurfactants, organic acids and biopesticides among others. (Abu Yazid et al., 2017).

2.1 AGRICULTURAL INDUSTRIAL WASTE:

Enzymatic hydrolysis is a common method for converting agricultural wastes into valuable goods. Wheat bran, husk, corncob, wheat straw and bagasse are some of the most inexpensive and accessible renewable carbon sources for such production of industrially important enzymes. Enzymes have a wide range of uses in industrial operations. (Fig.2). Different species of microorganisms from agricultural wastes have been used to manufacture and colour (Bharathiraja et al., 2017).

Table 1: List of potential uses of organic wastes and bioproducts with added value.

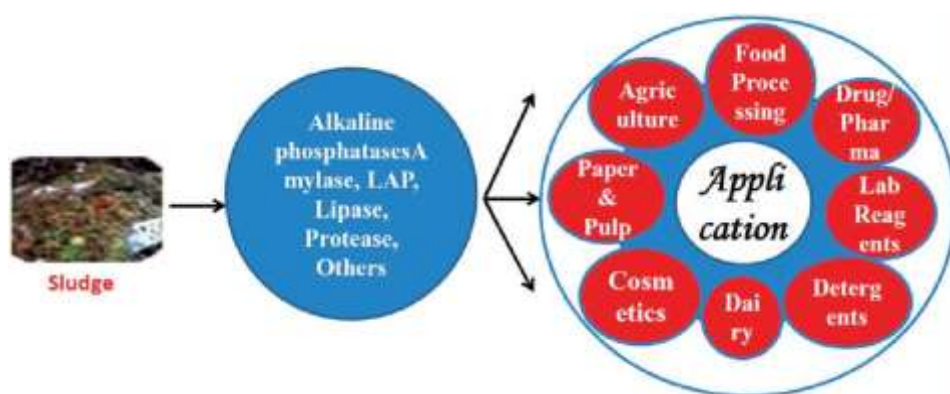
Organic waste	Type of Products Processes	Waste products	Utilization	References
Domestic/municipal food waste	Kitchen waste	food waste, sludge waste and cocoyam peel wastes	organic acids, Animal feed, antibiotics and biopesticides	Ohkouchi and Inoue,2007
Agricultural organic waste	Wheat, barley, Rice, soy, coffee, Sugarcane, corn	Peels, pomace, seeds, peels and husks	Animal feedstuff, enzymes, biofuel, chemical feedstock, organic acids and biopolymers,	Govumoni <i>et al.</i> 2015
	Veggies and Fruits		Compost, animal feed, enzymes and biofuels	
	Oil seeds and oils	Husks, sludge, presscake, fibers and shells	Biofuels, enzymes, bioethanol, biofertilizers, activated carbon	Jorgensen <i>et al.</i> 2010
Microbial Sludge Waste	Waste Water	Sludge	Protease	Karn et al., 2013
Food processing industrial waste	Waste water	Activated Sludge	Protease	Nabarlatz et al., 2012
	Sugar Production	Molasses	Enzymes, fructo-oligosaccharides	
	Poultry processing	Fats in skin, hairs, liver, bones, feathers, intestines, organ trim	Animal feedstuff, enzymes, biofertilizers	Jayathilakan <i>et al.</i> 2012
	Processing of Spices, cereals, vegetables, fruits	Stalks, chaff hull, husks, skin peels, pomace, seeds, residues fiber, stones	Activated carbon, organic acids, biofuels, Pectinolytic enzymes, animal feed.	Embaby <i>et al.</i> , 2014

According to Mihailovski et al. (2016), molasses and sugar beet peel stimulate *Paenibacillus chitinolyticus* CKS1 to generate higher α -amylase under ideal circumstances (3 % concentration of sugar beet pulp, 10% concentration of at an incubation of 88.07 hours 83.07 hours). De Castro et al. (2014) investigated the effects of agro - industrial wastes on the continual generation of protease and α -amylase under SSF. It was revealed that the wheat bran achieved the higher protease and α -amylase enzyme. Each year, huge quantities of agricultural waste are generated throughout the world. Cultivating microorganisms on agricultural residues as feedstock for the production of industrially significant products such as enzymes, polysaccharides, organic acids, aroma and flavour chemicals is a useful and promising strategy.

2.2 BACTERIOLOGICAL SLUDGE WASTE

As an outcome, in recent decades, effective waste disposal techniques are 3R (Reduce, Recycle, Reuse) methods to retrieve value-added products from both urban and industrial sludge, including such hydrolases. Enzymes for economic use are frequently derived from such a range of sources, notably animal cells, plant species and microbial sources (Lee et al., 2014).

Anbazhagan and Palani, (2018) reported that the consortia of hydrolytic enzymes such as α - amylase, protease, cellulase, lipase and α -glucosidase was extracted from Municipal Returned Waste Activated Sludge (MRWAS) using ultrasonication. Furthermore, the above mixture was stirred with TX100 (TritonX100) and AOT. The different proportions of



MRWAS and Dioctyl sodium Sulphosuccinate were reported.

Fig. 2. Source, enzyme and its application

Table 2: Recovery of enzymes from sludge

Enzyme	Sludge	Reference
Protease	Activated sludge	Karn et al., 2013.
Hydrolytic enzyme	Sludge	Nabarlatz et al., 2012
Protease	Sludge	Nabarlatz et al., 2010
Protolytic enzyme	Sludge	Adav et al., 2009
Lipase	Activated sludge	Karn et al., 2013.
Lipase	Anaerobic (Dairy)	Leal et al., 2006
Lipase	Anaerobic activated Sludge	Gessesse et al., 2003
L-Leu-aminopeptidase protease alanine-aminopeptidase	Anaerobic activated Sludge	Watson et al., 2004

Karn et al. (2013) examined that the activity of protease and lipase were closely

interconnected with the microbes in activated sludge than that of extracellular enzymes. The Triton X-100 which is non-ionic detergent for the extraction of greater protease and lipase using activated sludge at different concentrations. From these results, it was confirmed that the Triton X-100 extraction is an efficient methods to recover and maintain the lipase and protease. This research lays the groundwork for extracting enzymes from other industry.

2.3 HOUSE HOLD WASTE

Domestic sewage is a major source of pollution, particularly in Indian society. Household waste water is immediately released into ponds as well as rivers which leads to water pollution. Based on the survey data, it was found that there are not enough techniques are made to treat the waste water. Some of the kitchen waste in every day is comprises of vegetable, fruit and onion peel. Disposal of these waste is also found as complicated problems. Long time of these waste causes odor smell which causes health illness. From the kitchen waste, garbage enzymes are synthesized which a versatile liquid and it is used in the home, agriculture, animal husbandry and gardening, among others. Since, this enzyme is a protein-chained organic molecule with a complicated structure (Melikoglu et al., 2015).

3. INDUSTRIAL ENZYMES FROM ORGANIC SOLID WASTES AND THEIR APPLICATIONS

3.1 AMYLASE

Amylase is a hydrolase enzyme that converts starch molecules into glucose and maltose units. Amylases are classified into four different classification based on their enzyme activity α - amylase (1, 4- -D-glucan glucanohydrolase), β -amylase (1, 4- -D-glucan maltohydrolase), glucoamylase (GA) (1, 4- -D-glucan glucanohydrolase), also known as γ -amylase and debranching enzymes. Alpha amylases (EC 3.2.1.1, 1, 4--D-glucan glucanohydrolase) can hydrolyze internal-1, 4 glycosic linkages in amylose, starch, and amylopectin, converting them to maltose and glucose. More than 30% of amylase enzyme are produced using microbes (Far et al., 2020). Solid state fermentation (SSF) and Submerged fermentation SF are used to synthesize α -amylase. When compared to SF, SSF have several merits such as higher yield, water requirement and more energy utilization (Sethi et al., 2016). Recently RSM's Box-Behnken design to optimize the source for α -amylase generation is *Aspergillus oryzae*, which yield 9868.12 U/gds α -amylase. Further optimization of the important parameters revealed that an best conditions α -amylase synthesis is 64% of water content, 32°C temperature, 4.5 pH, 108 hour incubation time which was mentioned in the table 1 (Balakrishnan et al., 2021).

3.2 PROTEASES

Proteases are peptide-based enzymes that degrade proteins and it's divided into four types dependent on its activity namely acid, alkaline, thiol and metallo protease. Recent study was done to produce the protease enzyme from agricultural waste using *Bacillus subtilis*. It was found that higher protease yield was observed in groundnut oil cake around 334.17 U/m due to the influence of Ca^{2++} , Mg^{2++} , and Mn^{2++} . This protease enzyme synthesized from agricultural wastes exhibits enhanced catalytic and stability activity performs optimum pH found to be 7-8 and temperature 40°C (Elumalai *et al.*, 2020). Interestingly, the *C. limosum* strain was used to generate metalloprotease from animal meat at pH 6, 40°C, with a maximal specific activity of 220.9 U/mg. Chimbekujwo *et al.* (2020) revealed that protease enzyme produced from orange peel as a carbon source and yeast as a nitrogen source using the *Aspergillus* strain performs optimum at pH ranges of 4-6 and temperatures of 30-40°C.

3.3 LIPASES

Triacylglycerol hydrolases E.C. 3.1.1.3 (Lipase) is the potential classes of functional ingredients in biotechnological industrial process due to their attractive features for hydrolysis of fatty acid ester linkages in liquid media and synthesize them in non-liquid medium (Aguieiras *et al.*, 2017). The lipase enzyme was generated by *Bacillus sp*, which was collected from contaminated waste palm oil and exhibited highest enzyme activity at a pH 7 and temperature 37°C (Hasan *et al.*, 2018). The first study for the synthesis of lipase from wheat bran and cacao butter as substrate. The characterization showed the stability in the presence of EDTA, metal ions and surfactants (de Azevedo *et al.*, 2020). Lipase enzyme is used for pharmaceutical, esters formation, biodiesel, surfactant and treatment of wastewater (Naik *et al.*, 2018).

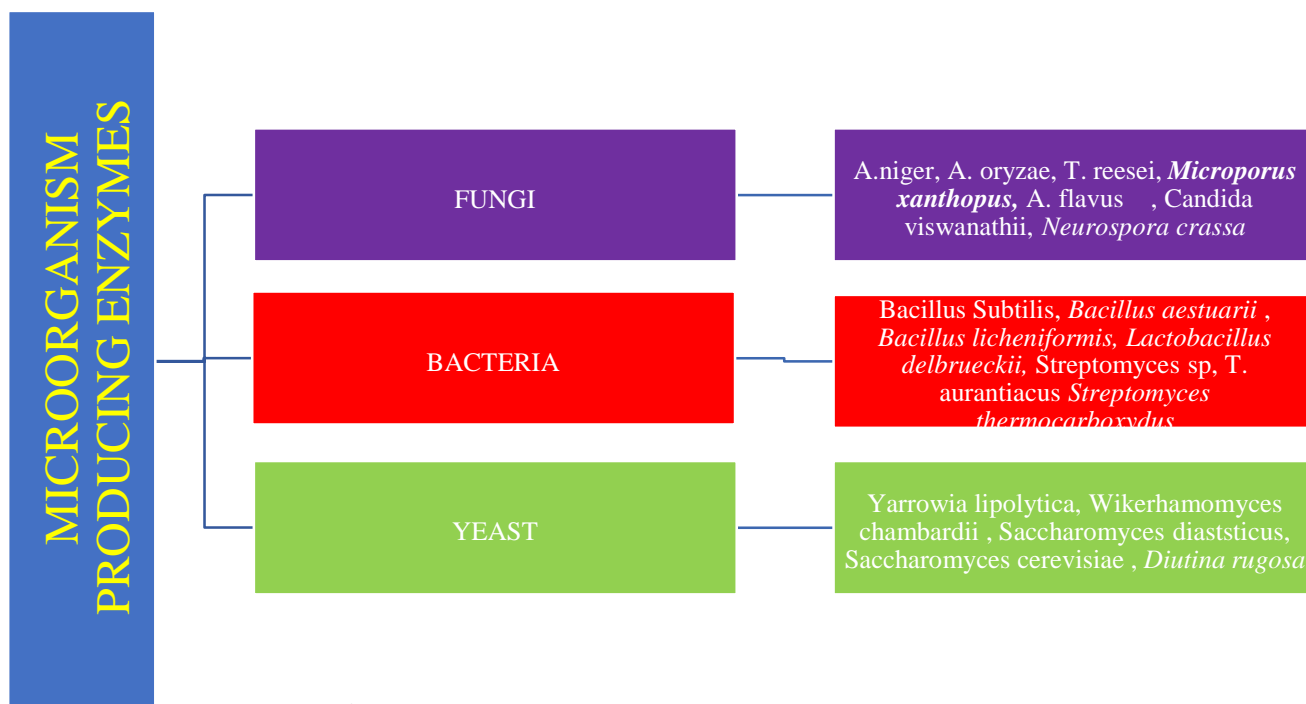


Fig. 3. Organisms used for enzyme production.

3.4 CELLULASE

The depolymerization of cellulose into glucose molecules a combination of enzymes: endoglucanase, exoglucanase or cellobiohydrolase and β -glucosidase. They belong to the family of glycoside hydrolases and catalyze the cleavage of glycosidic bonds (Juturu and Wu, 2014). Cellulase is an enzyme with important commercial significance. Especially because they play a key role in bioethanol synthesis (Singhania et al., 2014). In addition to biofuels, cellulose is also used in various industries, including brewing, textiles, bread, detergents paper and cellulose (Ferreira et al., 2014).

4. ADVANCED TECHNOLOGIES FOR ENZYME PRODUCTION FROM ORGANIC SOLID WASTES

4.1 SOLID STATE FERMENTATION

Solid state fermentation is a bioprocess technology applicable in various industries. SSF as a process involves microorganism cultivation on solid substrate with minimal moisture content. For the growth of microorganisms that produce enzymes, SSF uses solid support, biomass, and additional sources of carbon and nutrients. (Carboue et al., 2018). The selection of the substrate, the microbe, the pretreatment and particles size of the substrate, the moisture content, the type and size of a inoculum, the operating parameters also including temperature, pH, cultivation duration, oxygen consumption, etc. are all

included in SSF. (Krishania et al., 2018). SSF is a promising technique that employs agroindustrial waste as a substrate to generate enzymes. SSF is a cost-effective way to generate industrialized enzymes with modest and abundant substrate and high specific activity using organic waste.

4.2 SUBMERGED FERMENTATION

Batch, fed batch, and continuous submerged fermentation are all using liquid media for the development of microorganisms. The production of lipase in submerged culture was optimized using successive experimental design. Several optimized systems were applied in the fermentative situation. *A. flavus* lipase production with wheat bran as the main component of the culture medium, yeast extract as a nitrogen source at a concentration of 45 g/L, and soybean oil as a catalyst at a pH of 7.15 was one of the ingredients used to produce the perfect conditions. A mathematical model that used a central compound design to increase the pH concentration and yeast extract produced actual data that correlated with the model 94.18% more closely. (Colla et al., 2016).

VALORIZATION OF POTENTIAL ORGANIC SOLID WASTE INTO ENZYMES/BIOCATALYST FOR SUSTAINABLE INDUSTRIAL APPLICATIONS

Section A-Research paper

TYPE OF WASTE	MAJOR COMPONENTS OF WASTE	TYPES OF ENZYME	OPTIMUM CONDITION			MICROBIAL SOURCE	PRODUCTION RATE	APPLICATION	REFERENCES
			pH	TEMP °C	HR				
Groundnut oil cakes	Carbon, nitrogen, cellulose, lignin and hemicellulose	α -amylase	4.5	32.5	108	<i>Aspergillus oryzae</i>	9868.12 U/gds	Industrial application	Balakrishnan et al., 2021
Bread Waste	carbohydrate 49/100 g db starch 47.2/100 g db)	Protease and Amylase		30	120	<i>Rhizopus oryzae</i>	(100 U/g) and protease (2400 U/g	Industrial application	Benabda et al., 2019
Wheat bran with potato peel	Starch- cellulose, lignin and hemicellulose	α -amylase		37	96	<i>Bacillus amyloliquefaciens</i>	1.3 U/ug	Synthesis of silver nanoparticles	Mojumdar and Deka ,2019
Pearl millet	Starch,carbohydrate,calcium,protein	α -amylase	7	30	96	<i>Aspergillus terreus</i>	19.19 \pm 0.9 Ug ⁻¹		Sethi et al., 2016
Sugarcane molasses	Carbon source- cellulose, lignin and hemicellulose	Lipase and single cell protein	6	28		<i>Yarrowia lipolytica</i>	16420 U/ml of lipase and 151.2g/L	fodder purpose and fish feeding	Yan et al., 2018
rice bran with glycerol	Carbohydrates ,Ash , Fiber,Proteins,C/N	lipase	6	28	168	<i>Aspergillus niger</i>	(19.844 U·g-1)		Costa et al., 2017
brewery wastes	moisture,ash, carbon,crude protein FAN=free amino nitrogen in soluble fraction, in glycine equivalent	protease	6.5	37	3	<i>Lactobacillus delbrueckii</i>	2.6 U/mL		Mathias et al., 2017
pomelo peels	Carbon source	pectinase		30	168	<i>Aspergillus niger</i>		juice and food industry	Jalil and Ibrahim 2021
Dates fruit waste	Carbon source	pectinase	7	37	72	<i>Bacillus licheniformis</i>	5 g/dl		Aslam et al., 2020
Sugarcane bagasse waste	Cellulose,hemicelluloses, lignin	cellulase and xylanase	7	30	336	<i>Aspergillus flavus</i>	1.27 U/ml and 376.81 U/ml	food, fuel, textiles, pulp and paper industries	Namnuch et al., 2020
Sugarcane bagasse waste	Cellulose,hemicelluloses, lignin	xylanase	6.5–7.0.	60	48	<i>Bacillus aestuarii</i>	833.33 IU ⁻¹ h ⁻¹		Rashid et al., 2020
Spent coffee waste	Hemicellulose	Xylanase		30	120	<i>Aspergillus niger</i>		Fruit Juice enrichment	Ravindran et al., 2019

wheat bran	carbon source- cellulose, lignin and hemicellulose	xylanase	9	37	48	<i>Streptomyces thermocarboxydus</i>		Dye absorbent	Tran et al., 2021

Table 3 : List of organic waste, components and optimum conditions for Enzyme production and its applications

SMF was expensive and the productivity was less compared to solid state fermentation. Fungal strains are used in submerged fermentation which requires less water content. The steps involved in the overall process was illustrated in (Fig.4)

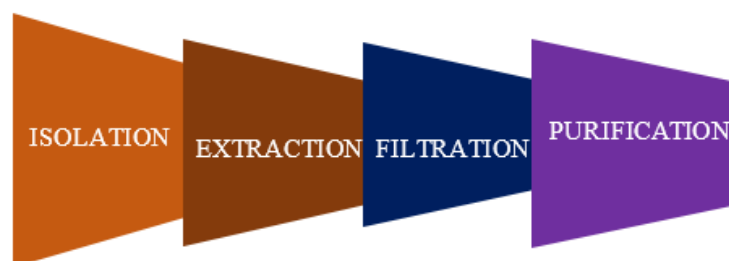


Fig.4. Steps involved in enzyme production

5. MICROORGANISMS FOR CONVERSION OF ORGANIC SOLID WASTE TO ENZYMES

Microorganisms used for the production of industrial enzymes were mentioned in (Fig.5) and it was discussed below. *A. niger* was recently isolated from multiple varieties of oil seeds for the synthesis of lipase. The ideal lipase production parameters were 40 °C, pH 7.5, 1% fructose as the source of carbon, 1% yeast extract as the source of nitrogen, 2% palm oil, 2.5×10^7 spores/mL solution, and 3 days of incubation. (Alabdallal et al., 2020).

- A. *niger* LFP-1 isolated using orange peels was utilized to produce pectinase. The purified pectinase had a molecular mass of 48 kDa. The ideal pH value was 3.5, and the ideal temperature was 50°C. The industrial biotechnological process, particularly in the fruit drinks and food business, utilizes the biochemical properties established by the pectinase enzyme in a variety of biochemical reactions (Jalil and Ibrahim, 2021). Solid waste from municipalities is utilized to generate beneficial microorganisms. *Pseudomonas stutzeri*, a strain with strong amylase activity, was discovered in municipal waste. The strain that had maximum activity at pH 7.5 and 40°C was employed to isolate the amylase enzyme. The strain is employed in the management of solid waste because it can tolerate harmful metal ions (Dutta et al., 2016).

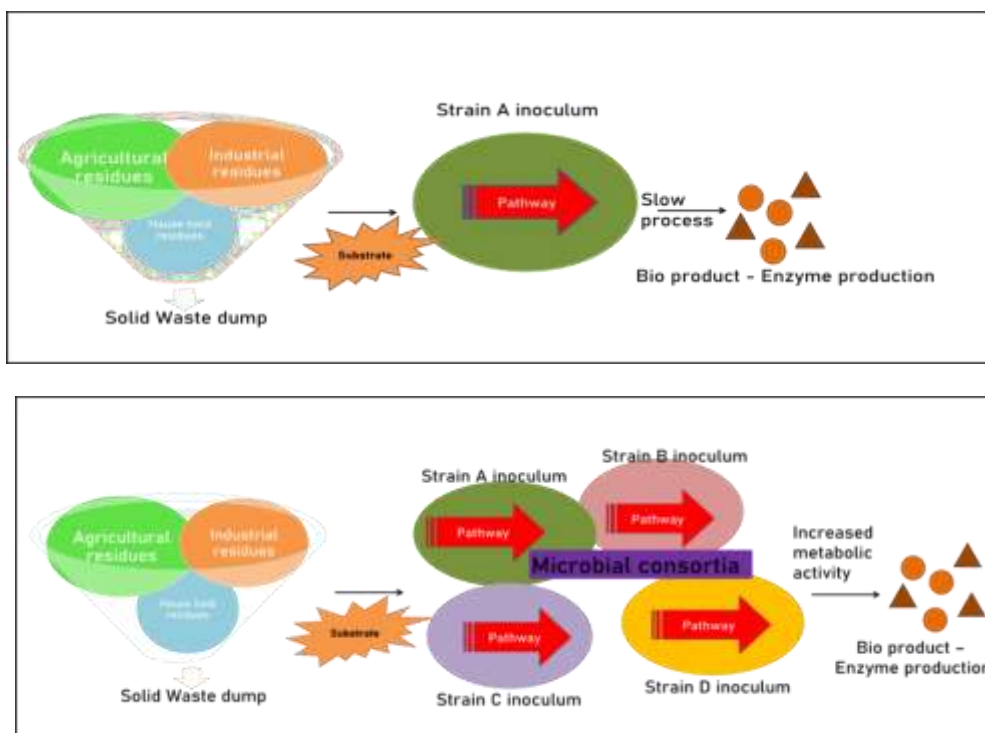


Fig. 5. A schematic description of a microbial consortium for bio productions

6. CONCLUSION

Organic Solid waste are big risk to the environment due to its large amount of generation. Utilization of these residues to a value-added product such as enzymes have been most appreciable. Usage of enzymes in all industry such as food, paper, textile, leather, and pharmaceuticals increase the demand. Most common methods to produce enzymes are SSF and SMF. Optimization of various parameters are done by Box behken and Response Surface Methodology. The microorganisms include bacteria, fungi, yeast and combination strains. Genetic manipulation improves the productivity and specific activity of enzymes. To produce enzymes, carbon sources derived from organic residues are used, resulting in a reasonable and scalable process. Purification process is critical. SSF is cost effective compared to SMF.

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