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Pullulan From Agricultural Waste Using *Aureobasidium pullulans* and Its Applications: A Review

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ABSTRACT

Pullulan is an extracellular homopolysaccharide commercially produced by the yeast-like fungus *Aureobasidium pullulans*. The interest in pullulan produced from industrial waste has significantly increased due to its applications in many industries. Pullulan has extensive applications in the cosmetics, pharmaceutical, and agricultural sectors. The primary limitation in the pullulan production is its high cost and limited yield. This made pullulan less useful for a variety of purposes. This study aims at the scope, limitations and future challenges in production of pullulan by using microbial sources. It will support more study on the application of diverse substrates, microorganisms, and their use in industrial sectors. This review will help to focus the interest on production of this complex polysaccharide and conduct experiments for the optimization of production parameters of the same. This may help to achieve a promising solution to the extraction of this polysaccharide overcoming the challenges and limitations.

1. INTRODUCTION

1.1 Carbohydrate

The term "carbohydrate" is a group of molecules that includes starch, sugars, and cellulose (Avenas, 2012). A broad range of polymers made of carbohydrates, viz. bacterial cellulose, xanthan, levan, dextran, gellan, pullulan, curdlan, kefiran, scleroglucan, etc. are produced by microbes. Pullulan is a homopolysaccharide that is linear and serves as the foundation for maltotriose units (Nasrollahzadeh *et al*. 2021)

Polysaccharides are generally hydrocolloids and water-loving polymers. These hydrocolloids have applications in food, pharmaceutical and cosmetic industries, because of their physical and chemical properties are distinct. They are used as a stabilizer, thickener, film-forming agent and emulsifier in the food products such as sauces, beverages, and, breads

etc. (Pirsa and Hafezi, 2023). They can be utilized in the pharmaceutical sector to make hydrogel tablets, hard capsules, and matrices. As polysaccharide-based gels are made from plant and animal sources, researchers have been looking for alternatives because of their simple availability for human consumption and lack of renewability (Kostag and El Seoud, 2021).

1.2 History

In 1958, Bernier made the initial discovery of carbohydrate pullulan from *Aureobasidium pullulans*. In 1959, Bender *et.al*., conducted a structural analysis of new polysaccharides and gave it the name pullulan. The fundamental makeup of pullulan was discovered in the 1960s (Abhilash and Thomas, 2017).

1.3 Pullulan

Polysaccharide pullulan is a linear, biodegradable, extracellular, and water -soluble homopolysaccharide produced by yeast -like polymorphic fungus *Aureobasidium pullulans* (Singh *et al*. 2019). Pullulan develops as an amorphous, slimy mass in the outer regions of the fungus *Aureobasidium pullulans*. It is a linear macromolecule that is are composed of repeating maltotriose units linked to the α-(1,6) glycosidic linkages (Li *et al*. 2015). The pullulan has a unique structure that makes it highly water soluble and biodegradable, so that this pullulan is a biologically functional macromolecule with great development values and application prospects (Ates, 2015). Pullulanase enzyme is a well-known, efficient debranching enzyme that is responsible for describing the structural attributes of pullulan. When Pullulanase enzyme hydrolyses pullulan, then the yield is several oligosaccharides, like maltose, maltotriose, maltotetrose, isomaltose, and panose (Rajeeva *et al*. 2010). Maltotriose is the primary repeating element of the pullulan structure. Chemical reactions may modify the pullulan structure to improve its physical characteristics.

Pullulan is a tasteless, odourless, and edible biopolymer that is not toxic, non-mutagenic, or non-carcinogenic. Pullulan is commonly used in a variety of industries, including pharmaceuticals, food, cosmetics, biomedical industries, targeted medication delivery, waste bioremediation agents, and medical (Sugumaran *et al*. 2017).

1.4 Structure of Pullulan

Pullulan's chemical formula is $(C_6H_{12}O_5)_n$, and the structure of pullulan is characterized by repeating maltotriose units, in which three glucose molecules are connected by α-(1→4) and α-(1→6) glycosidic bonds. Its spectral data approves the existence of both α-(1→4) and α-(1→6) linkages in pullulan.

Bernier was the first scientist to confirm that the pullulan structure contains glucopyranose (Bernier, 1958). The presence of α-(1→4) link was proved by investigating its optical rotation and infrared spectrum (Bender *et al*. 1959). Then, a few researchers discovered the structure of pullulan by employing infrared spectroscopy, methylation reactions, periodate oxidation and demonstrated the simultaneous existence of both α-(1→4) and α-(1→6) glycosidic bonds in a 2:1 ratio (Shingel, 2004; Sowa *et al*. 1963). (Singh and Saini, 2012). With the help of a hydrolytic enzyme called pullulanase, the pullulan structure can be investigated. The α-(1→6) glycosidic linkages serve as the specific site of pullulanase action on pullulan, resulting in its complete hydrolysis and the generation of maltotriose units (Hii *et al*. 2012). Maltotriose is formed by three α-(1→4) bonded glucopyranose units interconnected via an α-(1→6) glycosidic bond i.e. - [(1→4)-α-D Glucopyranosyl-(1→4)-α-D-Glucopyranosyl-

(1→6)-α-D-Glucopyranosyl]-. Alternatively, the incomplete hydrolysis of pullulan results in the formation of panose, maltose, isopanose and isomaltose (Bender *et al*. 1959; Sowa *et al*. 1963). Figure 1. Molecular structure of pullulan.

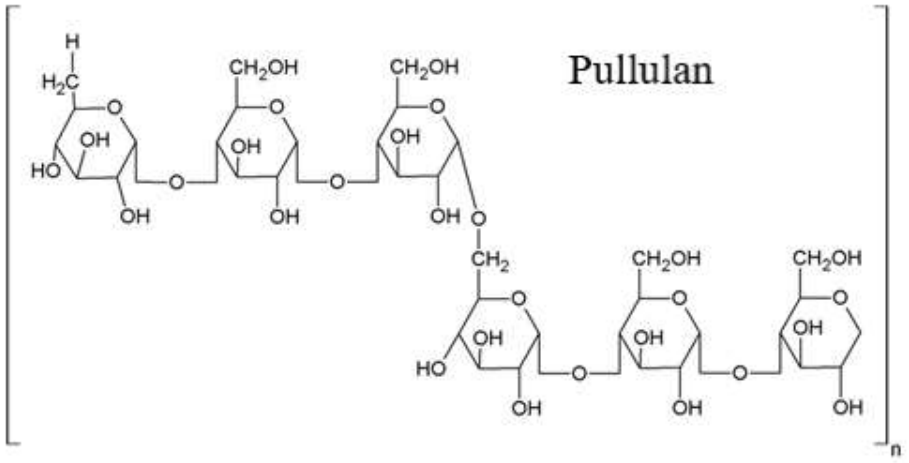

Fig 1: Molecular structure of pullulan (Ferreira *et al*. 2015)

1.5 Microbial Pullulan Producers

Pullulan was initially produced through microbial fermentation using the fungus *Aureobasidium pullulans* for commercial applications. However, not all strains of *A. pullulans* have been characterized for this purpose (Ghimici and Constantin, 2020). Instead of *Aureobasidium pullulans*, additional microbial strains can produce pullulan with differing ratios of glycosidic bonds, quantities of maltotriose units, and percentages of α-(1→6) linkages within the pullulan framework. A few strains which can produce a pullulan, including *Cytaria darwinii, Cryphonectria parasitica, Aureobasidium mangrovei*, *Cytaria harioti, Tremella mesenterica, Aureobasidium melanogenum, Rhodotorula bacarum, Teloschistes flavicans, Eurotium chevalieri,* and *Rhodosporidium paludigenum.* Table 1 provides fungal sources, structural components and their applications of pullulan.

Table 1: Fungal sources, Structural components and their applications of pullulan

Sr. No.	Fungal sources	Structural units	Applications	References
1.	*Aureobasidium pullulans.*	α-(1→4) linked glucopyranose rings interlinked by α (1→6) linkages.	Biomedical industry; Cosmetics industry; Food industry; Health oral care products, etc.	(Al-Araimi *et al*. 2021); (Basumatary *et al*. 2022); (Ghimici and Constantin, 2020) (Singh *et al*. 2008)
2.	*Aureobasidium mangrovei.*			
3.	*Aureobasidium melanogenum.*			
4.	*Cryphonectria parasitica.*			
5.	*Cyttaria darwinii.*			
6.	*Cyttaria harioti.*			
7.	*Eurotium chevalieri*			
8.	*Rhodosporidium paludigenum*			
9.	*Rhodotorula. Bacarum.*			
10.	*Teloschistes flavicans*			
11.	*Tremella mesenterica.*			

1.6 Pullulan Properties

Pullulan generally appears as a fine powder ranging from white to off-white in colour. The morphology of pullulan particles observed under microscopy indicates a smooth surface texture (Li *et al*. 2018; Soni *et al*. 2018). The unique, highly flexible structure of pullulan is responsible for its improved hydrodynamic properties (Singh *et al*. 2008). Pullulan's solubility is increased, and its solvent resistance is thus improved. Pullulan quickly dissolves in diluted alkali, both cold and hot water. Though pullulan is insoluble in organic solvents except dimethyl formamide and dimethyl sulfoxide. Pullulan is a dry, non-hygroscopic powder with a moisture content close to 10-15% without any stickiness. Pullulan can form viscous solutions, but it can't create gels. Pullulan solution shows non-Newtonian behaviour and shear-thinning behaviour. The shear stress versus shear rate plot of the pullulan solution exhibits a linear relationship. Table 2 and Table 3 show pullulan properties and solubility properties of pullulan.

Table 2: Pullulan properties (Viveka 2023)

Parameters	**Specification**
Physical state	Powder
Appearance	Whitish or yellowish-white
Flavour	Tasteless and odourless
Toxicity	Non-hazardous and non-cancerous
Molecular weight (kDa)	100 – 250
Consumability	Safe to consumption
Biodegrading nature	Biodegradable
Flow properties	Shear thinning and Non – Newtonian behaviour
Polypeptides (%)	Maximum 0.5
Solubility in organic solvent	Insoluble in all organic solvents with the exception of formamide and dimethyl sulfoxide
pH of solution range	5–7
Polydispersity ratio	2.1 – 4.1
Moisture (%)	10 -15
Mineral residue ash (sulfated, %)	Maximum 3
Viscosity range(cP)	80 – 180
Optical activity	Greater than ±160
Range of Melting point (°C)	250°C – 350°C

Table 3: Solubility properties of pullulan (Viveka 2023)

Solvent Name	**Solubility Conditions**
Water	Soluble
DMSO (Dimethyl sulfoxide)	Soluble
Pyridine	Slightly soluble
Ethyl acetate	Insoluble
Acetone	Insoluble
Dimethylformamide (DMF)	Partially soluble
chloroform	Insoluble

1.7 Gene Expression and Regulation

High pullulan-producing strains, such as *A. melanogenum* ZH27, show upregulation of genes linked to pullulan biosynthesis (UGT1, Ags2), glycolysis, and by-product synthesis (e.g., trehalose, glycerol, fructooligosaccharides). This coordinated gene upregulation is associated with both increased pullulan yield and enhanced cell resistance to osmotic stress (Wang *et al*. 2023)

In *A. pullulans*, transcriptomic analyses reveal that biosynthetic genes (upt, pgm, ugp, pul) are tightly regulated, and negative regulators like creA can suppress pullulan synthesis, especially under specific genetic backgrounds (e.g., melanin-deficient mutants) (Chen *et al*. 2023).

1.8 Morphological and Cellular Changes

Omics-guided studies have linked the presence of swollen cells with large vacuoles to periods of rapid pullulan accumulation, suggesting a physiological adaptation that supports high-level production (Wang *et al*. 2023).

1.9 Genetic Engineering and Mutagenesis

Targeted genetic modifications, such as knocking out genes involved in melanin synthesis or plasma membrane ATPases (PMAs), have been shown to alter both the quantity and molecular weight of pullulan produced. For example, deletion of PMAs in *A. pullulans* BL06 led to a 1.7-fold increase in pullulan titer but also a reduction in its molecular weight (Chen *et al*. 2023). UV mutagenesis and flow cytometry-based selection have also been employed to isolate high-yielding mutants, as confirmed by omics approaches (Zhang *et al*. 2024).

1.10 Environmental and Regulatory Factors

Transcriptome analysis under different growth substrates and environmental conditions (e.g., pH, carbon source) has identified regulatory factors such as pH transcription factor, which has a significant role in upregulating pullulan biosynthetic pathways (Wang *et al*. 2024; Zhang *et al*. 2023).

1.11 Metabolic Pathways and By-Product Formation

Omics studies have highlighted the balance between pullulan synthesis and by-product formation. While by-products like trehalose and glycerol help cells manage osmotic stress, their accumulation can distract carbon flux away from pullulan, impacting overall yield. Table 4 shows omics Insights into Pullulan Production.

Table 4: Omics Insights into Pullulan Production

Omics approach	**Key Findings**	**References**
Transcriptomics	Upregulation of biosynthetic and stress-resistance genes in high-yield strains	(Wang *et al*. 2023; (Chen *et al*. 2023; Wang *et al*. 2024)
Functional Genomics	Gene knockouts (e.g., PMAs, melanin) affect yield and polymer properties	(Chen *et al*., 2023; Zhang *et al*. 2024)
Morphological Studies	Swollen cells with large vacuoles linked to high pullulan accumulation	(Wang *et al*. 2023)
Regulatory Networks	pH and carbon source regulation via transcription factors like Appacc	(Wang *et al*. 2024)

1.12 Status of Pullulan Production in the Commercial Sector

Hayashibara Company Limited in Japan pioneered the commercial production of pullulan in 1976 and obtained patents for its commercialization. Hayashibara Company Limited is a leading producer of food-grade and deionized pullulan, offering products with molecular weights between 100 and 200 kDa (Agrawal *et al*. 2022).

Several different industries initiated commercial production of pullulan after Hayashibara's patent expired. Hayashibara Co. Ltd. is still recognized as the largest commercial pullulan supplier across the globe (Coltelli *et al*. 2020). Listerine is a brand name for oral care products that are marketed and commercialized by Hayashibara Company in partnership with Pfizer Inc. in the USA (Trinetta and Cutter, 2025). Currently conducting business in regions including North and South America, Europe, Asia, Southeast Asia, and Oceania. Hayashibara Industries is now known as Nagase Co. Ltd. The pullulan demand is gradually increasing due to its unique characteristics and potential uses of pullulan.

A comprehensive summary of the pullulan market conditions and their industrial applications can be found in a recent report named "Pullulan Market Research Report." Pullulan is predicted to have a global market value of USD 89 million by 2028, up from an estimated USD 68 million in 2022. Pullulan is expensive and in high demand worldwide, so it is necessary to assess the pullulan polymer's production process for industrial use.

Another well-known industry that produces pullulan commercially and supplies it for a significantly lower price of US $130/kg is Carbosynth Ltd. in the UK. Pullulan has also begun to be produced commercially by a number of Indian companies, including Jiyan Chemicals & Pharmaceuticals, Indexim International, Otto Chemie Pvt. Ltd., Kumar Organic Products Ltd., Thermo Fisher Scientific Pvt. Ltd. and Shreeji Pharma International.

In India, Kumar Organic Products Ltd. ranks among the leading producers of pullulan. Pullulan of the pharmaceutical grade is made in India by Jiyan Chemicals & Pharmaceuticals. Several other industries in China have also begun to produce pullulan on a commercial scale. viz. Shanghai Longyu Biotech Co., Ltd.; Shandong Hanjiang Chemical Co., Ltd.; Haihang Industry Co., Ltd.

The main global suppliers of pullulan among China-based companies are Shandong Jinmei Biotechnology Co. Ltd., Shandong Freda Biotechnology Co. Ltd., MeiHua Holdings Group Co. Ltd., Hebei Henbo Bio Technology Co. Ltd., and Shandong Kangnaxin Biotechnology Co. Ltd.

Several other U.S.-based companies, including Santa Cruz Biotechnology Inc. and MP Biomedicals Inc., have also entered the commercial production market for pullulan. According to Fact.MR projections, the global pullulan market will expand from USD 714.5 million in 2025 to USD 1,163.8 million by 2035, achieving a 5.0% CAGR over the forecast period (Pullulan market size and share forecast outlook 2025 to 2035, 2025).

The biggest market for pullulan, accounting for about 40.74% of global consumption, is the pharmaceutical industry. With a 36.24% share in pullulan consumption, the food industry ranks as the second largest market segment. Japan leads the consumption regions, with an annual usage of 667 million tons recorded between 2015 and 2019. China follows as the second largest market, holding a 20.65% share, while the USA accounts for 20.16% of global consumption volume. It is widely believed that the global potential for pullulan usage far exceeds the current scale of its commercial production.

1.13 Scope and Original Contribution of the Review

While recent reviews have covered pullulan production, applications, and waste valorization, they typically address substrate selection, fermentation optimization, strain engineering, and downstream processing as disconnected elements. This review stands apart through its holistic, decision-focused framework that systematically links waste substrate properties, fermentation outcomes, key bottlenecks, and omics-driven strain improvement.

Special attention centres on critical barriers like melanin pigmentation, excessive broth viscosity, substrate variability, and purification economics that determine industrial viability but receive fragmented coverage elsewhere. Unlike post-2020 reviews that mainly catalog experimental data, this synthesis emphasizes comparative evaluation and forward-looking strategies for scale-up. Through cross-study yield comparisons across substrates, fermentation systems, and strains, plus integration of techno-economic and biorefinery considerations, it exposes persistent gaps: scarce pilot-scale data, limited cost modeling, and poor omics-process synergy. This targeted analysis delivers a practical roadmap for sustainable, cost-competitive pullulan production from agro-wastes, extending beyond conventional literature surveys.

2. METHODS FOR PULLULAN PRODUCTION

A qualitative literature review approach was chosen to address the review's objectives to comprehensively explore and synthesize the diverse and complex information related to the production of pullulan from agricultural waste using *Aureobasidium pullulans*. The steps carried out for the review are shown in Figure 2.

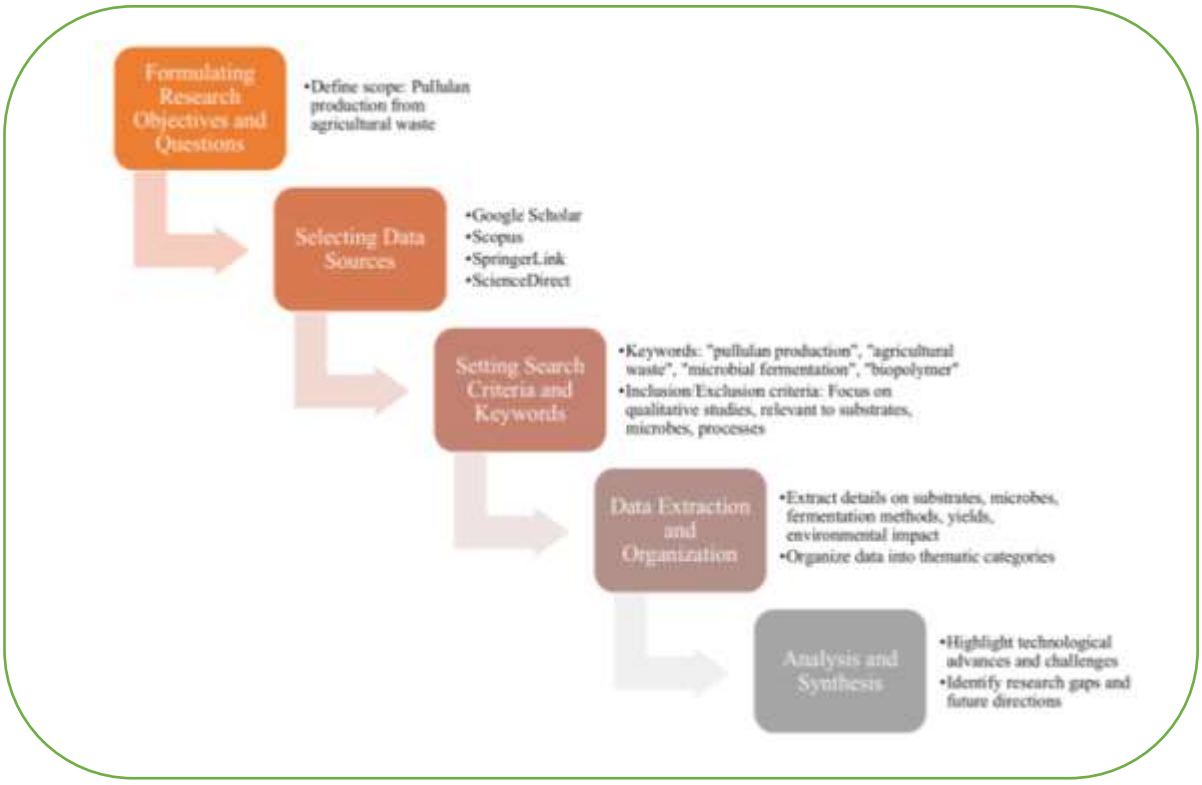

Fig 2: The steps carried out for the review

2.1 Data Collection and Literature Review

The literature review on the pullulan production from agricultural waste using *Aureobasidium pullulans* focused on collecting and synthesizing studies that explore the use of various agro-industrial residues—such as sugarcane molasses, potato starch hydrolysate, citrus peels, and hazelnut husks—as low-cost substrates for fermentation. Data were gathered from reputable scientific databases, including Scopus, Google Scholar, and SpringerLink, using targeted keywords like "pullulan production," "agricultural waste," and "*Aureobasidium pullulans*." Studies were selected based on their relevance to qualitative insights into fermentation processes, substrate optimization, and sustainability. The review highlights that employing agricultural wastes as fermentation media not only decreases production costs but also addresses environmental concerns by valorizing waste streams. Research consistently demonstrates that *A. pullulans* efficiently convert diverse agricultural residues into pullulan through both submerged and solid-state fermentation, with substrate type and composition significantly influencing yield and process efficiency.

2.2 Inclusion and Exclusion Criteria

Studies qualified for inclusion if they

- Demonstrated pullulan production from agro-industrial or agricultural waste substrates,
- Utilized *Aureobasidium pullulans* or related pullulan-producing microorganisms,
- Provided experimental fermentation data on performance metrics, substrate conversion, yields, or optimization strategies, or
- Addressed technological, economic, or sustainability implications of waste-based pullulan processes.

Exclusion criteria encompassed studies that

- Examined only pure/synthetic substrates without waste valorization relevance,
- Offered inadequate experimental or methodological details,

- Comprised conference abstracts lacking full-text access, or
- No direct connection to pullulan production.

The literature survey primarily covered studies published between 2000 and 2024, ensuring inclusion of both foundational and recent research. Title and abstract screening identified relevant studies, followed by full-text assessment. From approximately 200-300 screened publications, over 100 articles met criteria for comprehensive qualitative synthesis based on data quality and relevance. Discrepancies across studies were reconciled through comparative analysis, accounting for substrate variations, pretreatment techniques, strain differences, and fermentation parameters, preserving diverse perspectives to reveal critical trends, challenges, and research gaps.

Aureobasidium pullulans

Aureobasidium pullulans, previously identified as *Pullularia pullulans, Hormonema dematioides,* or *Dematium pullulans*, is an imperfect fungus. It is widespread in the natural environment and is commonly seen growing in soil and water, as well as on aged wood and various other plants. Taxonomists recently categorized *A. pullulans* as an anamorph of *Sydowia polyspora*, a teleomorph (Suzuki *et al*. 2021). Systematic classification of *A. pullulans* is shown in Table 5.

Table 5: Systematic classification of *A. pullulans*

Kingdom	Fungi
Phylum	Ascomycota
Class	Dothideomycetes
Subclass	Dothideomycetidae
Order	Dothideales
Family	Dothioraceae
Genus	*Aureobasidium*
Species	*Aureobasidium pullulans*

A. pullulans can consume various carbon sources such as mannose, glucose, sucrose, maltose, fructose, galactose, xylose and even the agro-industrial waste. The presence of isomerase and hexokinase is necessary for the carbon source to be converted to UDPG, which is an important precursor to synthesize pullulan (Sugumaran *et al*. 2017).

Aureobasidium pullulans has the capacity to produce melanin, so it is often referred to as "black yeast." Despite being a phytopathogenic fungus, *A. pullulans* do not produce any mycotoxins. According to Hermanides-Nijhof (1977), it is a ubiquitous, voracious saprophytic fungus that is frequently isolated from wood, water, soil, and other sources. The phyllosphere, toilets, optical lenses, food and feeds, deteriorating house paints, paper mills, hypersaline waters in salterns, hydrocarbons, synthetic materials, leather, human skin, watercolours, and the surface of decomposing leaves and other plant components have all been documented to contain *A. pullulans*. Because of its high rate of production and the distinctive qualities it produces, *Aureobasidium pullulans* has been selected for pullulan production. Additionally, this organism generates a variety of enzymes, including β-galactosidase, pectinase, amylase and xylanase (Viveka, 2023).

A. pullulans exhibit five distinct morphologies, including chlamydospores, mycelia, swollen blastospores, young blastospores, and yeast-like cells. The morphology of the fungus

determines the pullulan yield, though it is unclear which morphological form of *A. pullulans* is responsible for pullulan production (Oguzhan and Yangilar, 2013). One of the fungus's characteristics is the formation of dark-colored chlamydospores, and colonies initially appear smooth before becoming covered in a slimy mass. Because of its capacity to produce the polysaccharide pullulan, *A. pullulans* is an industrially significant organism.

In mild conditions, on potato dextrose agar plates at room temperature, *Aureobasidium pullulans* normally grows in 7 days and forms colonies with a diameter of 1-3 cm. The colony is initially smooth, flat, slimy, and leathery, and it can be pink, white, yellow, or light brown in colour. The formation of chlamydospores causes the colony to turn brown or black as it grows. While mature colonies form a visible, smooth, thin-walled mycelium, young colonies display blastoconidia structures under a microscope. Lobed chains of thick-walled chlamydospores and straight conidia are characteristics of *A. pullulans* that can be used to identify it (Wei *et al*. 2021).

Genome Sequencing of *Aureobasidium pullulans*:

The genomes of four varieties of *Aureobasidium pullulans* have been sequenced, revealing genome sizes between 25.43 and 29.62 Mb, encoding 10,266 to 11,866 predicted proteins (Gostinčar *et al*. 2014). These genomes contain genes for:

- Degradation of plant material (various enzyme families)
- Numerous sugar transporters
- Potential degradation of plastics and aromatic compounds
- Synthesis of pullulan and siderophores (but not aureobasidin A)
- Stress tolerance (aquaporins, aquaglyceroporins, alkali-metal cation transporters, compatible solute and melanin synthesis, high-osmolarity glycerol pathway, bacteriorhodopsin-like proteins)
- All genomes have a homothallic mating-type locus (Gostinčar *et al*. 2014).

Proteomics and Extracellular Vesicles (EVs):

Proteomic analysis of EVs from *A. pullulans* identified 642 proteins, mainly associated with primary metabolism (Černoša *et al*. 2022). EVs also contained proteins from various synthesis pathways, indicating multiple biogenesis routes. Small RNA sequencing of these EVs revealed two potentially novel miRNAs.

Functional Omics Applications:

i. Omics approaches are used to study complex interactions between A. pullulans, fruit hosts, pathogens, and native microflora, especially in the context of postharvest disease biocontrol (Castoria *et al*. 2001).

ii. Genetic engineering and metabolic pathway analysis (e.g., overexpression of main enzymes in the tricarboxylic acid pathway) have been applied to enhance production of valuable metabolites (Qin *et al*. 2024).

Taxonomic Insights: Genomic differences among varieties are substantial enough to suggest reclassification into separate species: *A. pullulans*, *A. melanogenum*, *A. subglaciale*, and *A. namibiae* (Gostinčar *et al*. 2014).

A useful basis for tackling important commercial problems related to pullulan production is provided by the availability of genomic, proteomic, and extracellular vesicle data

for *Aureobasidium pullulans*. The identification of genes involving melanin synthesis, sugar transport, polysaccharide biosynthesis, and stress tolerance provides direct targets for strain engineering intended to increase substrate utilization efficiency and decrease pigment formation, which significantly increases downstream purification costs. The secretion of metabolic enzymes and regulatory chemicals that may affect polysaccharide export and broth rheology is shown by a proteomic study of extracellular vesicles. This suggests chances to modify pullulan molecular weight and viscosity by targeted metabolic regulation.

2.3 Carbonaceous waste

2.3.1 Potato starch

A cheap and widely accessible agricultural product is the potato. Potatoes are mostly made of starch with traces of sugar. The leftover potato starch from the potato crisps production and other potato-processing industries is typically in the form of a homogenous substrate that is typically devoid of unnecessary components (Chauhan *et al*. 2023). Potato-derived starch has been employed as an alternative carbon source in several industrial fermentation processes. Some strains of *Aureobasidium pullulans* have enzymes that break down starch, but they are more active against linear α-1,4-glucans than they are against any polysaccharides that have α-1,6 linkages. Consequently, the starch waste must undergo partial hydrolysis to be considered a viable substrate for the production of pullulan (Göksungur *et al*. 2011).

Normally, just before the microbial fermentation process begins, the starch should be hydrolysed to produce sugar. However, using a potato as the carbon source eliminates the need for the expensive β-amylase enzyme. This is mainly due to the significant amount of highly active β-amylase found in potatoes. Apart from that, the degree of starch or dextrose equivalent hydrolysis determines how much pullulan is produced. This shows the overall amount of sugar reduction expressed as a percentage of glucose. While glucose has a dextrose equivalent of 100, some unhydrolyzed starch has a dextrose equivalent of 0. The pullulan content of the agglutinating substances increased during the fermentation process by using the hydrolyzed starch waste as the substrate, and on day six, it reached more than 90% (w/w).

2.3.2 Olive oil waste

Phenolic compound-containing waste from olive oil mills is one of the primary phytotoxic effluents produced. Olive oil waste damages plants and contaminates the environment (Pinho *et al*. 2017). Waste from olive oil includes 23 glucose, proteins, and total sugars. These sugars help *Aureobasidium pullulans* grow in the production medium, which increases the pullulan yield. Although a large number of microorganisms can't survive in the presence of waste olive oil, primarily because of the presence of harmful phenol chemicals. Despite this, *Aureobasidium pullulans* can survive and generate pullulan when olive oil waste is present (Youssef *et al*. 1998).

2.3.3 Sugarcane bagasse

One of the main crops grown in large amounts in India is sugarcane. A lignocellulosic substance known as sugarcane bagasse is created when sugarcane juice is extracted, producing solid trash. About 250 kilograms of bagasse are created for every ton of sugar produced, for an annual production of 100 million tons. The primary components of sugarcane bagasse include organic matter, lignin, cellulose, hemicelluloses, and ashes (Rocha *et al*. 2015).

2.3.4 Carob pod

Most crops cannot thrive in the warm, humid climates where carob trees typically flourish. Because of their high tannin content, carob pods can be fed to animals. 2% pectin, 5%

hemicelluloses, 7% cellulose, 20% phenolic components and 50% sugars make up the majority of carob pods. Because of these elements, extract from the carob pod has been utilized as a strong carbon substrate in submerged fermentation to produce pullulan (Palaiogianni *et al*. 2022).

2.3.5 Sweet potatoes

Sweet potatoes are root vegetables used for microbial fermentation that are primarily made of starch. Proteins, carbohydrates, lipids, vitamins, and ash are also present. Sweet potatoes are high in starch, but microbes find it difficult to use them. After the saccharification process, the hydrolyzed sweet potato can be used as a useful carbon substrate (Dewan *et al*. 2013). Fructose, maltose, glucose, maltotriose and other trace sugars are present in sweet potato hydrolysate.

2.3.6 Brewery waste

After wort and spent grains are separated, brewing firms release spent grain liquor, a waste liquid product. It contains 12 % cellulose, 40 % hemicelluloses, 13 % lipids, and 3 % ash. High biological oxygen demand, which is regarded as a primary contaminant, is present in spent grain liquor. Its biological oxygen demand will harm aquatic life when released into water supplies and pollute the ecosystem (Chaitanyakumar *et al*. 2011).

2.3.7 Rice hull

Rice is widely recognized as a basic essential food in various countries. The outer layer of rice grains, or rice hulls, is produced in huge quantities by the rice processing industries. The majority of the lignocellulosic materials found in rice hulls are cellulose (50%) and lignin (30%). The composition of rice hulls makes them suitable for use as a carbon substrate for microbial fermentation (Huang and Lo, 2019). Microorganisms are unable to directly utilize lignocellulosic sugars, so a saccharification procedure is needed to change this complex into fermentable sugars. After the pre-treatment process, rice husk hydrolysate can be utilized as a suitable carbon substrate for the synthesis of pullulan.

2.3.8 Corn steep liquor

A by-product of the maize wet-milling industry, Corn steep liquor is commonly used as a nutrient source for the production of various microbiological products. This by-product is mainly applied as a nitrogen source. Fungal pigment synthesis can be cultivated on waste stream cellulose generated from corn (Panesar *et al*. 2015). This waste, which is produced by processing corn and comes from the milling process, is employed as a non-synthetic input in the majority of liquid fertilizer formulations for the cultivation of organic crops. As reported by Chiani, *et al*. (2010), it typically contains 88 g of ash per kilogram of dry matter, 130–220 g of carbohydrates per kg of dry matter, 205 g of crude protein per kg of dry matter, 525 g of dry matter, and a trace amount of sulfurous acid (<0.01 g/kg DM). Additionally, 42% of CSL is protein (Chiani *et al*. 2010).

2.3.9 Other food industrial residues

Aureobasidium pullulans has effectively produced pullulan using a variety of food industrial residues as substrates. These by-products are rich in carbohydrates and other nutrients, making them suitable and cost-effective alternatives to refined sugars in fermentation processes. Table 6 shows the conversion of food processing by-products into pullulan using *Aureobasidium pullulans*.

Table 6: Conversion of food processing by-products into pullulan using *Aureobasidium pullulans*.

Processing food waste	**Fermentation process**	**Fermentation time, temperature etc.**	**Pullulan yield (g/L)**	**References**
Molasses	Shake and flask (Submerged fermentation)	5 days, 35 °C, 150 rpm,	45.00	(Srikanth *et al*. 2014)
Beet Molasses	Stirred tank bioreactor	5 days, 400 rpm, 28 °C, aeration rate 2 vvm	6.6	(Göksungur *et al*. 2004)
	Shake flasks fermentation		16.7	
Cassava bagasse	Solid-state fermentation	5 days, 30 °C	19.00	(Sugumaran *et al*. 2014)
De-oiled rice bran	Shake and flask (Submerged)	7 days, 30 °C, 150 rpm	54.8	(Singh and Kaur, 2019)
Potato starch water	Stirred tank reactor fermentation (Submerged)	5 days,28 °C, 500 rpm	54.57	(An *et al*. 2017)
Rice hull	Stirred tank reactor fermentation (Submerged)	3 days, 28 °C, 400 rpm, aeration rate 3 L min^{-1}	22.20	(Wang *et al*. 2014)
Sesame seed oil cake	Solid-state in flask	25 °C, 2 h, 200 rpm	54.50	(Mirzaee *et al*. 2020)

Although diverse food and agro-industrial wastes have successfully supported pullulan production, yields varied substantially across substrates and fermentation modes (Table 6). High-performing substrates de-oiled rice bran (54.8 g/L), potato starch water (54.6 g/L), sesame seed oil cake (54.5 g/L), and molasses (45.0 g/L) delivered superior yields attributable to their abundant fermentable carbohydrates and balanced nutrient profiles, requiring minimal pretreatment. Conversely, beet molasses produced markedly lower yields (6.6-16.7 g/L) in bioreactors, likely due to its sucrose-dominant composition, inhibitory compounds, and shear stress at high agitation rates (400-500 rpm) that impair exopolysaccharide biosynthesis by *Aureobasidium pullulans*.

Furthermore, fermentation mode played a critical role, with solid-state fermentation (SSF) achieving superior yields (19.0-54.5 g/L) relative to submerged fermentation (SmF; 6.6-54.8 g/L) despite simpler process control; sesame seed oil cake excelled in SSF due to concentrated nutrient content and reduced dilution effects, while variations in fermentation time, temperature, and agitation influenced oxygen transfer, metabolic activity, and broth viscosity. These observations highlight that pullulan production efficiency is governed by the interplay of substrate composition, fermentation strategy, and process parameters rather than substrate type alone.

2.4 Hydrolysis of waste

Globally, both rural and urban areas in many developing countries, including India, face significant challenges related to solid waste management. In some cases, bioconversion of

these wastes—such as the microbial synthesis of different enzymes, organic acids, and feed—can be both economically advantageous and lessen environmental pollution (Rishi *et al*. 2020).

Agro-based industries produce a vast quantity of waste, and if the waste is discarded untreated, then it can result in significant environmental issues. However, these agro-industrial wastes are a rich source of nutrients, organic and inorganic matter. This waste can be utilized as an alternative carbon or nitrogen source for the production of various microbial products. It helps in reducing the environmental pollution generated by the direct removal of untreated waste and is also economically good (Singh *et al*. 2021).

Commercially relevant yields of glucose can only be obtained from the hydrolysis of cellulose in the presence of a catalyst. (Hu *et al*. 2015). There are three basic types of catalysts, which include enzymatic, concentrated acid, and diluted acid. The key benefits of employing enzymatic catalysts are their high specific characteristic that is, their lack of by-products, their ability to function in mild environments, their environmental friendliness, and the fact that a tiny amount of enzyme produces large yields. However, in order to use enzymatic hydrolysis, pre-treatment is required to free up the structure and provide the enzyme access to the active sites. Pre-treatment is frequently carried out with physical methods that require a lot of energy, high pressure and temperature, or the application of a chemical solvent, such as diluted acid. Additionally, lignin may block the hydrolysis of an enzyme. Long reaction periods, big reactors, and the high cost of enzymes are some additional drawbacks of enzyme hydrolysis (Orozco *et al*. 2007).

The only pressure involved in concentrated acid operations is that which is produced when materials are pumped from one vessel to another at comparatively low temperatures. The breakdown of sugars into unwanted byproducts is minimized by these low pressures and temperatures. The cellulose chain's hydrogen bonding is broken by concentrated acid, leaving it in an amorphous condition. After de-crystallization, the cellulose and acid combine to form a homogenous gel that permits hydrolysis reactions. The solution can then be diluted by adding water at low temperatures, which will create the ideal environment for glucose to develop (Orozco *et al*. 2007).

Dilute acid hydrolysis is usually carried out at a higher temperature range of 200°C–240°C, while a lower temperature is required for concentrated acid hydrolysis and higher glucose yields of about 90% is achieved using 30%–70% sulfuric acid (Niju *et al*. 2020). But concentrated acid hydrolysis requires a large amount of acid, causing corrosion to equipment, and the main drawback with concentrated acid is the huge cost of acid; a recovery step is necessary to lower the cost.

Because of its high production costs, pullulan has limited use in industrial applications despite its unique features and potential uses. Pullulan production requires a cost-effective strategy in order to increase its appeal for industrial uses. A few strategies have been employed to lower the price of producing pullulan, such as enhanced strains, genetic engineering advancements, and the discovery of low-cost sources of carbon and nitrogen. Thus, inexpensive substrates are crucial for creating processes that are both profitable and practical.

In a 2018 study, Mishra *et al*. compared the pullulan production to that of xanthan gum. They discovered that the cost of production of pullulan is three times higher than that of xanthan gum. Production of pullulan from raw material utilization accounts for about 30% of total production costs. Pullulan's unique structural properties probably necessitate a complex and expensive purification process, which accounts for the majority of its higher cost. In order to lower expenses and improve pullulan's economic feasibility for industrial applications, the study emphasizes the necessity for additional optimization of the production of pullulan

techniques. The price difference between xanthan gum and pullulan may be caused by economies of scale and decreased demand. The writers stress how crucial it is to deal with these financial aspects to encourage the widespread (Mishra *et al*. 2018).

The type of fermentation process, fed-batch or continuous, also has a big impact on the amount of pullulan generated by *Aureobasidium pullulans*. The pullulan production is also significantly affected by the fermentation conditions. The components in the fermentation medium are used by *Aureobasidium pullulans* cells during the fermentation process to produce pullulan and cellular biomass. The rheological response of the fermentation broth is influenced by the cellular biomass, cell debris, and pullulan accumulation that occur during fermentation. During the pullulan production, the rheology of the fermented broth changes from Newtonian to non-Newtonian. Increased viscosity in the fermentation broth reduces microbial cells to use oxygen and growth factors, and this leads to decreased productivity of pullulan during fermentation. Controlling various parameters during the fermentation process is necessary (Viveka, 2023).

Acid and enzymatic hydrolysis are common methods to break down agro-waste into sugars for pullulan production, but their cost-effectiveness varies in this context. Acid hydrolysis is cheaper and faster for sugar release, yet it produces inhibitors like furfurals and acids that harm *Aureobasidium pullulans* growth and pullulan yield, raising detoxification costs. Enzymatic hydrolysis provides better sugar quality, fewer inhibitors, and reliable fermentation, leading to consistent pullulan output despite higher enzyme expenses and slower times. For pullulan, enzymatic methods are preferable for process stability and product purity, while acid hydrolysis suits setups with detoxification or multi-product recovery. Few studies compare these economically for pullulan, pointing to a need for industrial-scale assessments.

2.5 Pullulan production through fermentation by using hydrolysed cellulosic waste

A fungus called *Aureobasidium pullulans* produces a commercially available carbohydrate polymer, pullulan. *Aureobasidium pullulans* cells produce pullulan as an exopolysaccharide in the late stages of its life cycle, and it builds up as slime on the cell surface. Various strains of A. pullulans have been documented to produce pullulan through fermentation. (Cheng *et al*. 2011a). For every strain of *Aureobasidium pullulans* to produce pullulan, a particular media composition and environment are needed. Production of pullulan is greatly dependent on the fermentation medium composition, specifically the nitrogen source, carbon source, pH and medium supplements of the fermentation medium. The type of fermentation system, batch, fed-batch, or continuous, also has a big impact on how much pullulan are produces by *A. pullulans* (Youssef *et al*. 1999).

The Pullulan production is significantly influenced by the fermentation conditions. During the entire fermentation process, *Aureobasidium pullulans* metabolizes components of the medium to form biomass and secretes pullulan. The medium changes from Newtonian to non-Newtonian due to the effects of cellular biomass, cell detritus, and accumulated pullulan during fermentation on the rheology of the fermentation broth. Because of the increased viscosity of the fermentation medium, pullulan production is less efficient because microbial cells are unable to use oxygen and growth nutrients as effectively. The production of pullulans by *Aureobasidium pullulans* can be increased by better controlling different aspects of the fermentation process. (Cheng *et al*. 2011b). Figure 3 shows the pullulan synthesis mechanism.

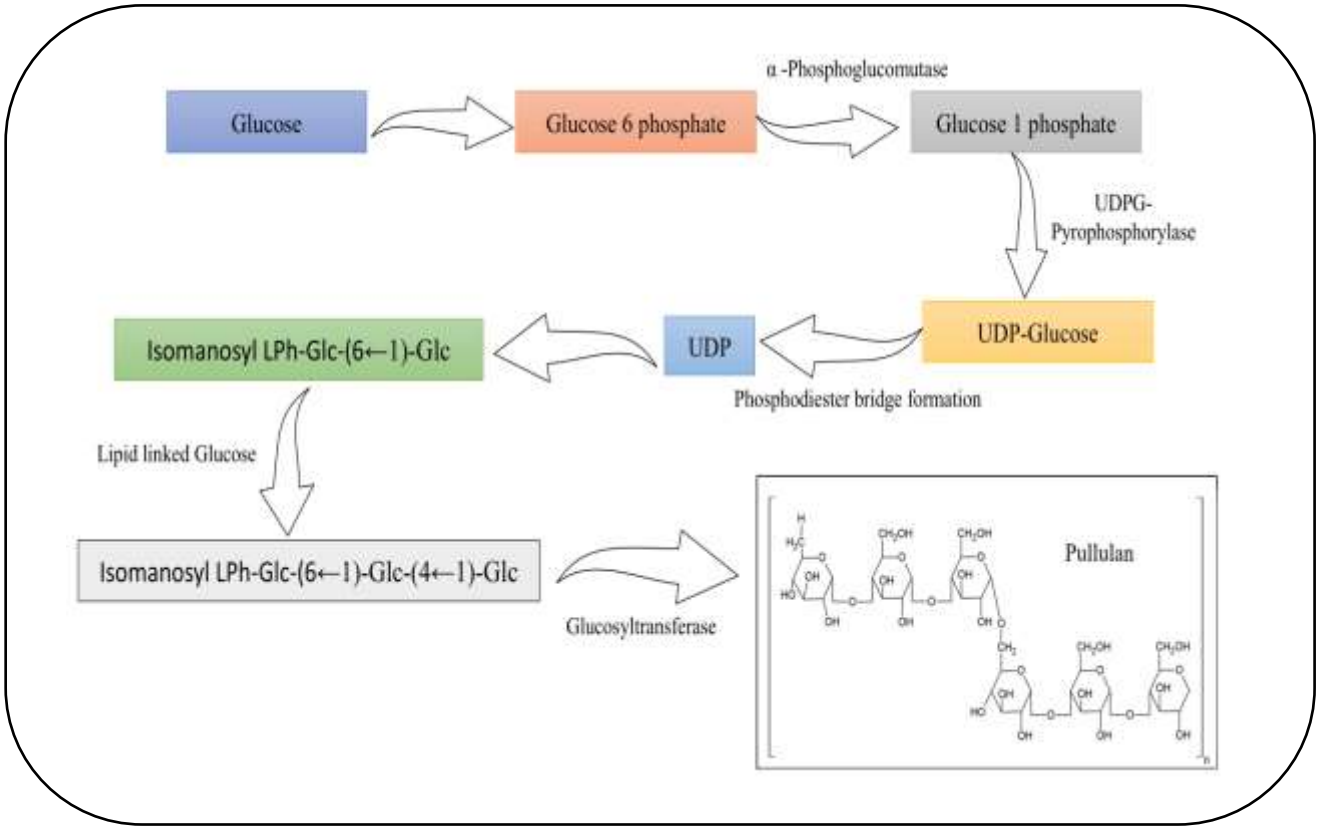

Fig 3: Pullulan synthesis mechanism. (Mishra *et al*. 2023)

2.6 Factors Affecting the Production of Pullulan

Production of pullulan is influenced by several variables, including inoculum size, age, temperature during fermentation, and medium pH. *A. pullulans* consume the medium ingredients during the fermentation process to produce pullulan and cellular biomass. By altering the fermentation broth's rheology, biomass and cell debris buildup also have an impact on the production of pullulan (Wani *et al*. 2021). A higher viscosity in the fermentation broth reduces the quantity of oxygen and medium components that *A. pullulans* can consume, which slows down the production of pullulan. Production of pullulan in shake-flask is greatly impacted by the fermentation medium's composition, including the nitrogen source, carbon source, medium supplements, and medium pH. Pullulan production is affected by the following parameters demonstrated as -

2.6.1 Temperature

The temperature during fermentation is a significant component in pullulan production. The optimum temperature for the growth of microbial cells ranges from 25–30°C, which starts the pullulan production during shake flask fermentation. While lower temperatures slow the growth of cells and pullulan yield, higher temperatures reduce pullulan yield. The ideal temperature range for the production of pullulan is between 28 and 30°C (Wu *et al*. 2012).

2.6.2 pH

One crucial element in the fermentation process is the pH of the fermentation medium. The pH of the strain changes according to the type of microbe being used. The parameters of the process and the formulation of the fermentation medium have an impact on the medium's pH. Variations in pH have a significant effect on the morphological features of the cell culture in the fermentation broth, which slows down the process of fermentation and results in less

pullulan being produced. According to the majority of research, a pH range of 5.0–7.6 is ideal for the growth of microbial cells and pullulan production (Gaur *et al*. 2010).

2.6.3 Time

Another crucial factor in the pullulan synthesis is the fermentation period, which is determined by the requirements of the microbial isolate. After being injected into the fermentation broth, the cultured cells adjust to their surroundings and begin the process of fermentation. Pullulan production and growth of cells take place during the log phase of cells. The cells enter the stationary phase as the fermentation continues, which inhibits the production of pullulan. Temperature and medium composition have an impact on how long pullulan takes to produce. Each strain has a different fermentation period. Under shake-flask fermentation, three to seven days is the optimum fermentation period for pullulan production (Hamidi *et al*. 2019).

2.6.4 Inoculum size

The term "inoculum size" refers to the number of microbial cells required to inoculate the fermentation medium to produce pullulan. When there are enough microbial cells, *A. pullulans* cells can grow to the necessary size for pullulan production. The yield of pullulan and cell biomass increases as inoculum size increases. Reduced yield of pullulan during fermentation is caused by insufficient cellular biomass growth from smaller inoculum sizes of microbes (Mirzaee *et al*. 2020). According to the reports, the optimum inoculum size for *Aureobasidium pullulans* to produce pullulan during shake-flask fermentation is 5% (Ding *et al*. 2020).

2.6.5 Inoculum age

One of the crucial factors influencing the pullulan production from *Aureobasidium pullulans* is the age of the inoculum. The most active form of *Aureobasidium pullulans* is yeast-like cells, which are normally used for inoculation (Jiang *et al*. 2018). When producing pullulan, microbial cells in log phase are typically preferred for inoculation over those in the lag phase. Fermentation times are shortened when the cells of *Aureobasidium pullulans* are in log phase. However, inoculating *Aureobasidium pullulans* cells during the stationary phase lengthens the process of fermentation and decreases the growth uptake factors needed to produce pullulan. According to published research, the best inoculum age for the production of pullulan is one to four days old (Haghighatpanah *et al*. 2021).

2.6.6 Agitation

In shake flask fermentations, agitation is important to the pullulan-producing process. The viscosity of the fermentation medium often rises during pullulan formation, which makes it difficult for the microbes to use the medium's ingredients (Kumar *et al*. 2019). During fermentation, the agitation keeps the medium homogeneous and helps in the constant mixing of its constituent parts. In a homogeneous medium, the microorganisms have constant access to oxygen (Lazaridou *et al*. 2002) and easy accessible to the medium's nutrients (Jafari *et al*. 2007). It promotes pullulan accumulation as well as the proliferation of microbial cells (Jafari *et al*. 2007; Lazaridou *et al*. 2002). A concentration gradient between the inside and outside of the cell, as well as a constant mass transfer across the cell wall, is also maintained by appropriate agitation (Choudhury *et al*. 2012). The type of microbe will also determine if agitation is necessary.

2.6.7 Carbon sources

The primary source of energy in the fermentation medium is the supply of carbon, which promotes the microbial cells' growth. There have been reports of the production of pullulan using a variety of carbon sources, including lactose, sucrose, galactose, glycerol, glucose, xylose, arabinose, cellobiose, rhamnose, inulin, fructose, and maltose (Singh *et al*. 2008). The majority of research work has shown that when sucrose is utilized in shake-flask fermentations as a carbon source, the maximum pullulan production occurs (Badhwar *et al*. 2018; Badhwar *et al*. 2019; Chen *et al*. 2018; Chen *et al*. 2020; Chen *et al*. 2020b; Haghighatpanah *et al*. 2021; Hamidi *et al*. 2019; Jiang, 2010; Jiang *et al*. 2019). During fermentation, sucrose is the most effective inducer of β-fructofuranosidase in *Aureobasidium pullulans* cells because pullulan is a non-reducing sugar. When the amount of sucrose in the fermentation medium is low, β-fructofuranosidase catalyzes the conversion of sucrose into glucose and fructose.

However, in the initial stages of fermentation, fructose and sucrose are bound together. β-fructofuranosidase also promotes the accumulation of ketose when the sucrose concentration is high (Sheng *et al*. 2016). In shake-flask fermentations, glucose is another common carbon source in the fermentation medium, which is used to produce pullulan (Cheng *et al*. 2011a; Gaur *et al*. 2010; Hamidi *et al*. 2019; Liu *et al*. 2018; Ma *et al*. 2014; Prasongsuk *et al*. 2005; Prasongsuk *et al*. 2007). The Embden-Meyerhof-Parnas pathway is used in *A. pullulans* biosynthesis to produce pullulan from glucose during fermentation (Fernandes *et al*. 2024). Less pullulan yield from glucose has been found in most investigations than from sucrose (Sheng *et al*. 2016).

2.6.8 Nitrogen sources

During fermentation, the nitrogen source controls the metabolic activity of the cells and affects pullulan formation (Wang *et al*. 2015). Production of pullulan has been reported using a variety of complex organic nitrogen sources, such as urea, peptone, yeast extract, and ammonium sulphate, as well as inorganic nitrogen sources, such as ammonium oxalate, ammonium formate, ammonium chloride, ammonium citrate, ammonium nitrate, ammonium acetate, copper sulphate, sodium nitrate, etc. In most shake-flask fermentations, it has been discovered that a combination of yeast extract and ammonium sulphate is the optimal source of nitrogen for *Aureobasidium pullulans* to produce pullulan. Pullulan manufacturing has also been achieved with the use of only yeast extract (An *et al*. 2019; Hamidi *et al*. 2019; Jiang, 2010). This is an extremely multifaceted organic nitrogen supply made up of many peptides, amino acids, vitamins, minerals and other substances.

2.7 Downstream processing of pullulan

A downstream processing is a multi-step procedure used to safely recover a microbial product while keeping its biological activity and purity. It must be effective, reproducible, and also maximise product recovery yield while requiring the least number of resources to produce (Singh, 2014). Selecting inexpensive or complex substrates to formulate fermentation media on an industrial scale typically results in lower upstream processing costs. On the other hand, the downstream process requires more steps to separate the inexpensive/complex substrates, which are highly impurity-filled, so it can raise the cost of the final product. However, using highly purified substrates in the fermentation medium reduces the complexity of downstream processing steps, but their cost is high.

Before downstream processing, the fermented medium may occasionally receive a pretreatment to preserve the microbial product and prevent its degradation (Svarovsky, 2001).

To fulfil the requirements for a certain microbial product's usage, various downstream processing processes are used to achieve the product's required level of purity. In general, it is best to reduce the steps involved in the downstream processing in order to reduce the loss of microbiological product at various steps of purification, shorten process times, and lower production costs.

The downstream treatment of pullulan includes liquid-liquid separations after solid-liquid separations. Conventional techniques for downstream processing of pullulan include precipitation using organic solvents, dialysis, ultrafiltration, gel filtration chromatography, etc. NMR spectroscopy, chromatographic techniques and FTIR spectroscopy are further used for the confirmation of pullulan structure (Shingel, 2002). Design of pullulan production from agricultural waste is shown in Figure 4.

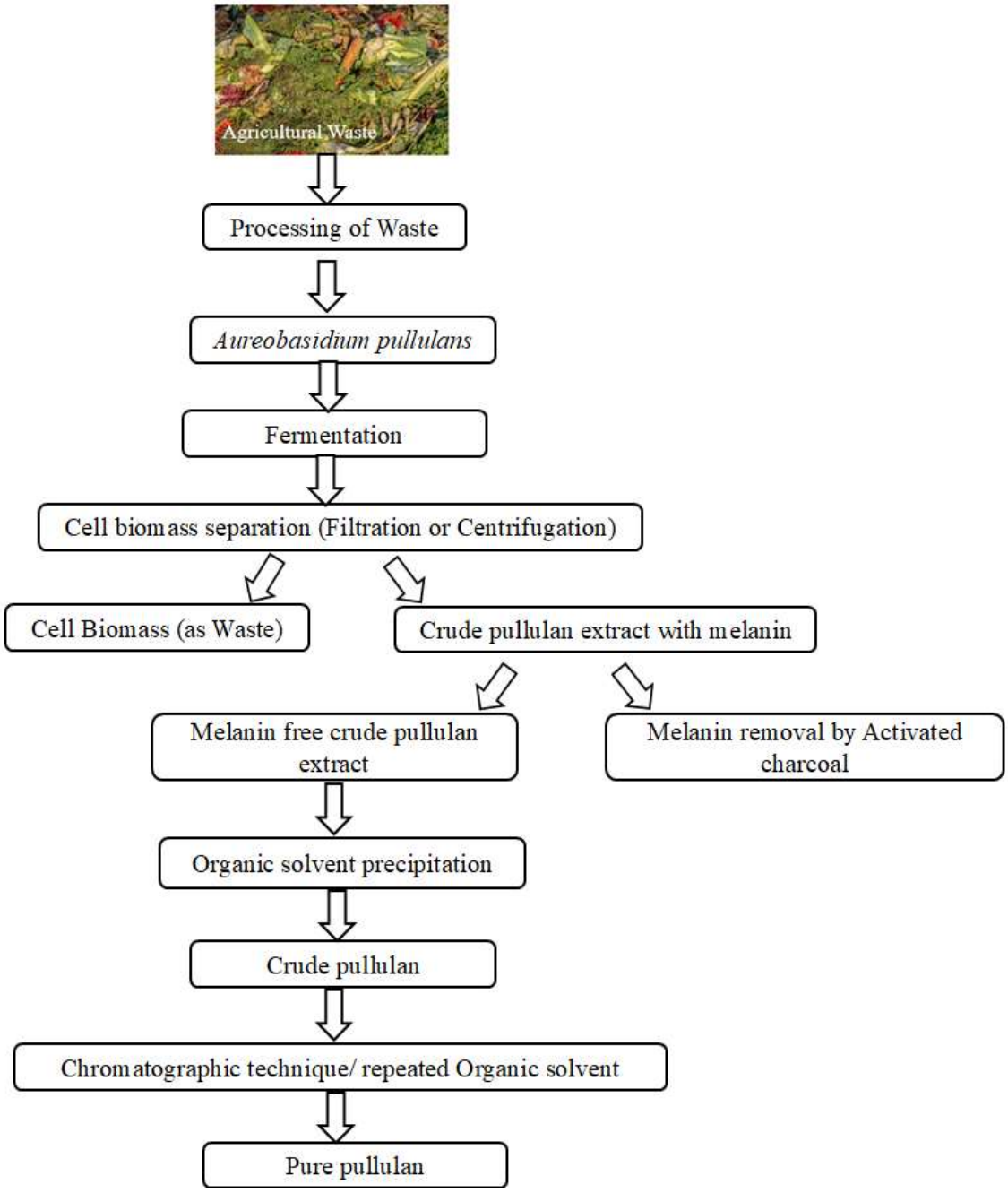

Fig 4: Design of pullulan production from agricultural waste (Singh *et al*. 2019)

3. PROBLEMS FACED DURING PULLULAN PRODUCTION

In spite of its unique properties, pullulan's high production cost often three times higher than that of other polysaccharides like xanthan or dextran, remains a critical barrier to widespread industrial adoption. This is due to several factors:

- High Cost and Complexity of Raw Material Preparation:

The high cost of nutrients and raw materials, even when using waste substrates, as well as the need for extensive pretreatment and hydrolysis for some residues (Cruz-Santos *et al*. 2023). This captures the dual challenge of expensive nutrients and the need for extensive pretreatment or hydrolysis when using certain agricultural or food industry residues as substrates for the production of pullulan. The cost of raw materials can account for up to 30% of total production costs, and the requirement for additional processing steps further increases overall expenses and operational complexity (Mehta *et al*. 2014).

- Downstream processing:

Downstream processing challenges, such as the high viscosity of fermentation broths and the co-production of melanin pigment, complicate purification and increase costs.

i. High viscosity of culture broth:

High viscosity of the broth is a big problem that has been faced during pullulan production. During the fermentation process, pullulan may degrade (pullulanolysis) only because of the production of melanin pigment. In the fermentation medium, the microbial cells, residual media components, cellular debris and extracellular metabolites are left after the fermentation process is completed. Hence, for the separation of these impurities alternative downstream process is needed before the precipitation of pullulan (Ray and Moorthy, 2007).

ii. Melanin production:

The pentaketide pathway is involved in the synthesis of melanin pigments and pullulan, both intracellularly and extracellularly. The medium composition and culture conditions affect the melanin synthesis. Activated charcoal is utilised to separate melanin (Kachhawa *et al*. 2003). However, the main disadvantage of activated charcoal is that, as a result of contamination, some pullulan and fine charcoal powders are lost. The viscosity of the broth is also increased by the introduction of activated charcoal. Therefore, it is necessary to look for another technique for separating the pigments in melanin. The need for colourless or low-melanin-producing strains is pressing, as melanin removal adds a costly step to the process.

4. INDUSTRIAL AND COMMERCIAL APPLICATIONS OF PULLULAN

Pullulan has several uses in the biomedical industries, food, cosmetics and pharmaceutical industries because of its special qualities (De Souza *et al*. 2023). Pullulan finds application in the pharmaceutical business as a packaging material. According to Tabasum *et al*. (2018), pullulan can also be used as a low-calorie polysaccharide in food packaging, dietary fiber, drinks, stabilizers, binders, and coating materials. Pullulan serves as a novel biomaterial in tissue engineering and various biomedical fields, and its derivatives have applications in gene targeting and medication (Tabasum *et al*. 2018).

Pullulan has various uses in different kinds of cosmetic products, such as cosmetic lotions, cosmetic powders, rouges, cosmetics for use around the eyes, facial packs, shampoos, set lotions, hair lacquers, and toothpaste (Roy *et al*. 2020). Pullulan's exclusive characteristics and physical features make it an ideal biopolymer for use in the cosmetics industry. The greatest

application of this polysaccharide is in oral care products. These products using pullulan are broadly commercialised (Singh and Saini, 2012). Due to its non-irritating properties, pullulan finds wide application in a range of cosmetic products, powders, hair styling, lotions, shampoos and facial masks. It is employed in mascara and eyelash products as a film-forming agent to increase lash length and volume, resulting in a more voluminous and defined appearance. Additionally, pullulan is employed in hair care items like gels, hair sprays and styling creams, where it acts as a film-forming and fixative agent, providing hold and improving the durability of hairstyles (Coltelli *et al*. 2020).

Pullulan-based skin care products are useful as a skin-whitening agent. It supports the tissue and also corrects the structural architecture, which is necessary for the regeneration of tissue (Xiao *et al*. 2012). Pullulan is incorporated into anti-ageing formulations, including firming lotions and wrinkle creams. It offers a temporary tightening effect, giving the skin a smoother and more youthful appearance (Chen *et al*. 2014).

The pullulan can be utilized in the next-generation point-of-care testing for storage of labile biomolecules as stable water-soluble pellets for a long time, and the drug capsules made of pullulan are warmly welcomed by vegetarians and Muslims, diabetics, and patients with restricted diet (Liu *et al*. 2017).

Cell division and cell growth mechanisms for skin renewal slow down as skin ages (Coltelli *et al*. 2020). A novel cosmetic formulation may incorporate an efficient delivery system capable of crossing the skin barrier and releasing active compounds at targeted skin layers. When an element is delivered to a certain layer, the intercellular connection is restored, and the ageing or damaged dermis structure is hydrated and renewed (Prausnitz *et al*. 2004).

Traditionally, the carriers for cosmetic products have been polymers with strong water solubility, such as carboxymethyl cellulose, alginate, hydroxyethyl cellulose, starch and methyl cellulose. Novel cosmetic products can also be made with natural polysaccharides as carriers (Danti *et al*. 2019; Morganti and Coltelli, 2019). According to Dubey and Kashyap (2018), it is wholly non-mutagenic, non-immunogenic, and non-carcinogenic in origin. It is very water soluble, tacky, low viscosity, and has a high moisture absorption rate. Pullulan's comparatively low viscosity makes it an excellent binder for cosmetic formulations (Parul and Dubey, 2018).

Pullulan is an element that can increase the quality of cosmetic products. Pullulan can be used in any amount in a cosmetic product without limitations due to its GRAS designation and lack of toxicity. Pullulan's photoprotective qualities have led to a rise in the demand for hair and skin care products rather than makeup with chemicals. Table 7 shows the properties of pullulan and its cosmeceutical properties.

Table 7: Properties of pullulan and their cosmeceutical properties (Coltelli *et al.* 2020)

Pullulan properties	Cosmetic application
White to off-white appearance, non-toxic, odourless, water-soluble, adhesive, and tacky	Used as an ingredient in liquid and paste rouges to improve texture and adhesion
Film-forming, safe and non-irritant	Ingredients in eyeliners and eye shadows for smooth application and skin compatibility
Moisturizing, adhesive, film-forming; retains continuity during peeling, with film shrinkage providing skin surface tension	Active component in facial packs to enhance skin feel and performance
Foam-enhancing and stabilizing properties	Used in hair shampoos to improve foam quality and stability
High coherence, viscosity, non-toxic, and foam stability during storage	Toothpaste ingredient for texture and foam consistency
Good water solubility contributes to transparent film formation	Applied to face masks and hair styling products for aesthetic and functional benefits
Strong film-forming ability, adhesive, hair-setting capacity, and ease of removal	Used in body lotions and hair lacquers for styling ease and product removal

CONCLUSION

Agro-industrial waste offers a sustainable, cost-effective carbon source for pullulan production owing to its abundance, nutritional value, and waste valorization potential. While extensive research validates pullulan synthesis from varied waste feedstocks, industrial scalability remains hindered by exorbitant downstream processing costs, primarily from melanin pigmentation, excessive broth viscosity, and inconsistent purity with waste substrates, which escalate purification demands and impair process economics. Among mitigation strategies, omics-informed strain engineering combined with precision fermentation control emerges as the most viable solution. Genomic, transcriptomic, and proteomic insights enable the development of *Aureobasidium pullulans* variants exhibiting minimized pigmentation, enhanced waste substrate conversion, and regulated polysaccharide molecular weight, complemented by process optimization for superior yields and consistency. Downstream technologies alone, however, fall short without concurrent upstream biological enhancements. Critical literature gaps persist, notably the absence of pilot-scale demonstrations, waste-specific techno-economic analyses, and holistic strain-process integration. Bridging these through multidisciplinary efforts is crucial to elevate pullulan production from lab prototypes to competitive circular bioeconomy processes.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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