

**Development and Characterization of Gelatin -
Based Bioplastic Derived From Fish Scale Waste**

A Research Project

Submitted by

AISHWARYA M. ANGAJ

SAMINA F. MULANI

KALYANI D. YADAV

UNDER THE GUIDANCE OF

DR. KOMAL K. BHISE

(Assistant Professor)

DEPARTMENT OF MICROBIOLOGY

VIVEKANAND COLLEGE, KOLHAPUR

(AN EMPOWERED AUTONOMOUS INSTITUTE)

YEAR 2025-2026

“Dissemination of education for Knowledge, Science and culture”

- Shikshanmaharshi Dr. Bapuji Salunkhe

Shri Swami Vivekanand Shikshan Sanstha's

VIVEKANAND COLLEGE, KOLHAPUR
(AN EMPOWERED AUTONOMOUS INSTITUTE)

DEPARTMENT OF MICROBIOLOGY (PG)

CERTIFICATE
OF
RESEARCH PROJECT COMPLETION

This is to certify that Ms. **AISHWARYA M. ANGAJ** studying in M.Sc. part II, Sem-IV at Vivekanand College, Kolhapur (An Empowered Autonomous Institute) has sincerely completed research project work entitled “**DEVELOPMENT AND CHARACTERIZATION OF GELATIN-BASED BIOPLASTICS DERIVED FROM FISH SCALE WASTE**” during academic year 2025-26.

Dr. Komal K. Bhise

Research Project Guide

Examiner

Dr. T. C. Gaupale

Head of the Department
I/C Head
Department of Microbiology
Vivekanand College, Kolhapur
(Empowered Autonomous)

“Dissemination of education for Knowledge, Science and culture”

- Shikshanmaharshi Dr. Babuji Salunkhe

Shri Swami Vivekanand Shikshan Sanstha's

VIVEKANAND COLLEGE, KOLHAPUR
(AN EMPOWERED AUTONOMOUS INSTITUTE)

DEPARTMENT OF MICROBIOLOGY (PG)

CERTIFICATE
OF
RESEARCH PROJECT COMPLETION

This is to certify that Ms. **SAMINA F. MULANI** studying in M.Sc. part II, Sem-IV at Vivekanand College, Kolhapur (An Empowered Autonomous Institute) has sincerely completed research project work entitled **“DEVELOPMENT AND CHARACTERIZATION OF GELATIN-BASED BIOPLASTICS DERIVED FROM FISH SCALE WASTE”** during academic year 2025-26.

Dr. Komal K. Bhise

Research Project Guide

Examiner

Dr. T. C. Gaupale

Head of the Department
I/C Head

Department of Microbiology
Vivekanand College, Kolhapur
(Empowered Autonomous)

“Dissemination of education for Knowledge, Science and culture”

- Shikshanmaharshi Dr. Bapuji Salunkhe

Shri Swami Vivekanand Shikshan Sanstha's

VIVEKANAND COLLEGE, KOLHAPUR
(AN EMPOWERED AUTONOMOUS INSTITUTE)

DEPARTMENT OF MICROBIOLOGY (PG)

CERTIFICATE
OF
RESEARCH PROJECT COMPLETION

This is to certify that Ms. **KALYANI D. YADAV** studying in M.Sc. part II, Sem-IV at Vivekanand College, Kolhapur (An Empowered Autonomous Institute) has sincerely completed research project work entitled “**DEVELOPMENT AND CHARACTERIZATION OF GELATIN-BASED BIOPLASTICS DERIVED FROM FISH SCALE WASTE**” during academic year 2025-26.

Dr. Komal K. Bhise

Research Project Guide

Dr. T.C. Gaupale

Head of the Department

I/C Head

Department of Microbiology
Vivekanand College, Kolhapur
(Empowered Autonomous)

ACKNOWLEDGEMENT

I wish to express my deep sense of appreciation to Dr. Komal K. Bhise, Assistant Professor, Department of Microbiology, Vivekanand College, Kolhapur (An Empowered Autonomous Institute) for her valuable support and expert guidance during the course of this study. She has been extremely understanding and cooperative and has always taken great interest in this work.

I wish to express my sincere thanks to Dr. C. B. Patil, Principal, Vivekanand College, Kolhapur (An Empowered Autonomous Institute) and Dr. T. C. Gaupale, Head, Department of Microbiology for providing the laboratory facilities in the department to carry out the experimental work.

I express my thanks to Ms. V. V. Misal, Coordinator of PG Department of Microbiology and faculty members, Dr. S. D. Mali, Ms. M. M. Nadkarni, Ms. S. S. Shaikh, Ms. A. T. Patil, and Ms. P. P. Patil for their valuable suggestions and help during the work.

I convey my gratitude to Mrs. S. N. Salokhe (Laboratory Assistant), Mr. S. K. Maskar and Mr. D. R. Patil (Laboratory Staff) of the department for their kind help in the laboratory.

I am thankful to the librarian and library staff for providing facilities of computer and reference books. My special thanks and gratitude to my entire classmates who have been constant source of inspiration and help during entire project work. I am highly obliged to authors past and present whose literature has been cited.

Finally, I thank my family members for their blessings and support because of which this work has proved satisfactory to me.

Place: Kolhapur

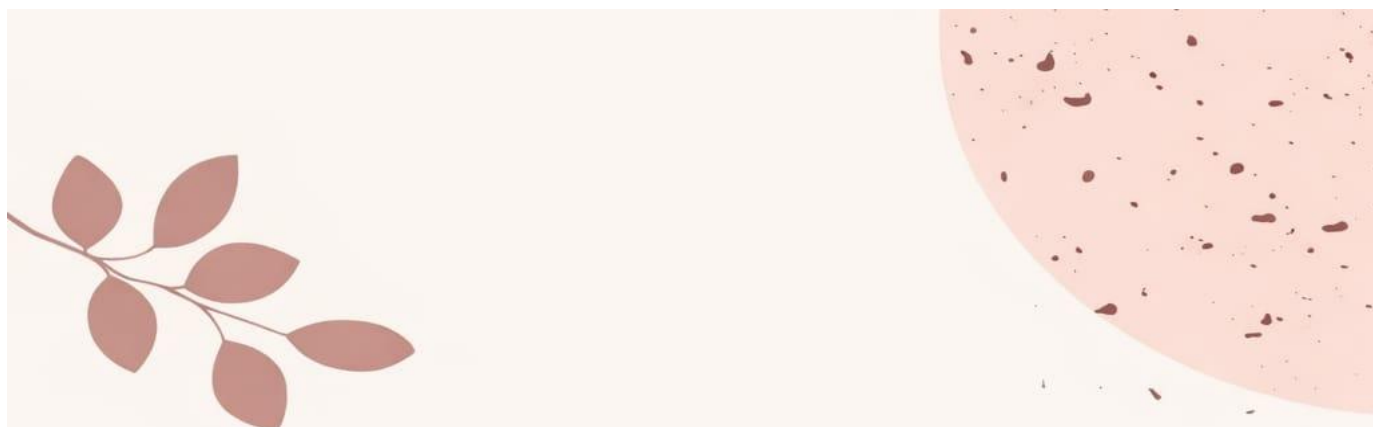
Date: 15/04/2026.

Ms. Aishwarya M. Angaj
Ms. Samina F. Mulani
Ms. Kalyani D. Yadav

Aishwarya...
Samina
Kalyani

INDEX

| Sr. No. | Title | Page No. |
|---------|--|----------|
| 1. | Introduction | 5 - 11 |
| 2. | Review of Literature i. Fish consumption And The Fish Industry ii. Gelatin iii. Food industry and cosmetic application. iv. Alternative source fish scale and other by products v. Economic impact of fish gelatin in production vi. Importance of gelatin produced by using fish waste vii. Advantages of fish waste gelatin viii. Research area for future studies ix. Plastic x. Bioplastic | 12-26 |
| 3. | Objectives | 27 -28 |
| 4. | Material and methods | 29-40 |
| 5. | Result | 41-66 |
| 6. | Discussion | 67-71 |
| 7. | Conclusion | 72-74 |
| 8. | Bibliography | 75-77 |



INTRODUCTION



INTRODUCTION

The global fish processing industry generates millions of tons of waste annually in the form of skins, scales, bones, viscera, and fins. These byproducts, if not properly managed, contribute significantly to environmental pollution and economic loss (Arvanitoyannis & Kassaveti, 2008). In many regions, such residues are discarded directly into water bodies or landfills, leading to increased biochemical oxygen demand, foul odour, and the proliferation of pathogens. Paradoxically, these so-called “wastes” are rich in high-value biomolecules such as collagen, chitin, proteins, lipids, and minerals (Gomez-Guillen et al., 2011). The fish processing industry produces substantial waste, with scales often discarded and underutilized despite their high collagen content, a primary source for gelatin production. Fish scales are a rich source of organic matter and are used in agriculture as organic fertilizers to improve soil fertility and plant growth by improving soil’s physical & chemical properties. (Gomez-Guillen et al., 2011).

India alone contributes nearly six million tonnes of fish annually, with the majority derived from the marine sector, while shrimp represents a substantial portion of national exports (Marine Products Export Development Authority [MPEDA], 2019). The global production of fish and shrimp has shown a consistent upward trend over the last few decades, with approximately 74% of the total yield being utilized directly for human consumption (Food and Agriculture Organization [FAO], 2020). However, during fish processing, nearly 60% of the fish biomass is discarded as waste, including heads, skins, bones, scales, and viscera (Arvanitoyannis & Kassaveti, 2008). This waste not only contributes to environmental challenges such as pollution, odor, and microbial proliferation but also represents a potential raw material for producing high-value bioproducts (Gomez-Guillen et al., 2011).

Fish processing byproducts such as skins, bones, and scales—which constitute about 30% of the total fish mass—can be effectively utilized in several industries to produce fish meal, fertilizers, collagen, gelatin, chitin/chitosan, enzymes, and even biofuels like biogas and biodiesel (Kim

& Mendis, 2006). The valorisation of such waste materials is vital for promoting environmental sustainability and enhancing the economic profitability of the fishery sector (Shahidi, 2020). Additionally, the utilization of fish waste reduces the overall biochemical oxygen demand (BOD) in water bodies and minimizes disposal costs for the seafood processing industry (Venugopal & Shahidi, 1995).

Among the valuable compounds derived from fish waste, gelatin has gained special attention due to its wide range of applications in the food, pharmaceutical and cosmetic industries. Gelatin is a partially hydrolysed form of collagen, which serves as a major structural protein in connective tissues, skin, and bones (Johnston-Banks, 1990). Traditionally, commercial gelatin has been produced from mammalian sources, primarily pigskin and bovine hides or bones. However, growing concerns about zoonotic diseases such as bovine spongiform encephalopathy (BSE), along with religious and dietary restrictions in Muslim and Hindu communities, have created a strong demand for alternative sources such as fish-derived gelatin (Karim & Bhat, 2009).

Fish gelatin exhibits unique physical and chemical characteristics compared to mammalian gelatin. It generally has a lower melting and gelling point, making it more suitable for cold food applications (Jamilah & Harvinder, 2002). Tropical and warm-water fish species have been reported to yield gelatin with rheological and textural properties comparable to bovine gelatin (Gudmundsson & Hafsteinsson, 1997). Furthermore, gelatin extracted from freshwater fish species provides an eco-friendly, non-religious, and sustainable alternative to conventional sources (Zhou et al., 2006).

Nevertheless, the production of high-quality gelatin from fish waste faces several challenges. The biochemical composition of fish collagen is less stable than that of mammals, leading to lower thermal stability and a higher risk of microbial degradation if waste is not processed immediately (Benjakul et al., 2012). Effective storage, rapid processing, and optimized pretreatment conditions (acidic or enzymatic) are therefore essential to ensure consistency in gelatin quality (Ahmad & Benjakul, 2011)

Gelatin:

Gelatin is a hydrocolloid widely applied in confectionery, dairy products, capsules, and cosmetic formulations due to its unique gelling, emulsifying, and stabilizing properties (Karim & Bhat, 2009). Traditionally, the majority of gelatin is produced from porcine and bovine sources through acid, alkali, or hot-water extraction of hides, bones, and skins. However, these conventional sources face several limitations. Religious restrictions in Islamic communities prohibit the consumption of pork-derived products, while Hindu traditions avoid bovine sources. Moreover, public health concerns such as bovine spongiform encephalopathy (BSE) and swine influenza have further reduced consumer acceptance of mammalian-derived gelatin (Karim & Bhat, 2009). Marine collagen and bovine collagen differ slightly in the types of collagen. As you might expect, collagen comes from fish -is found in fish skin and scales. If you eat the skin when you have a meal that includes fish, you're essentially getting extra collagen. Marine collagen supplements are those that contain collagen derived from these fish sources. They are therefore the preferred choice for pescatarians or anyone who eliminates beef from their diet (Karim & Bhat, 2009).

Gelatin is a protein obtained by the partial hydrolysis of collagen, a fibrous structural protein found in the connective tissues, skins, bones, and scales of animals. It exhibits unique functional properties such as gel formation, emulsification, foaming, and film formation, which makes it indispensable in the food, pharmaceutical, photographic, and cosmetic industries (Karim & Bhat, 2009). Beyond agriculture, fish scales are also processed for valuable materials, including: collagen for pharmaceuticals and cosmetics, chitin/chitosan for biodegradable plastics and water purification, and decorative pigments for paints and textiles. Their unique biocompatibility also makes them useful in tissue engineering and as biosorbents for treating wastewater. Marine collagen is also used to control bleeding. Different products are commercially available; they have sponge-like structures and are highly absorbent and able to hold many times their own weight of fluid. They may be cut to desired shape and applied to a bleeding surface. The placed product rapidly absorbs the blood, creating an

artificial clot-like structure that stops the bleeding at the site. (Daniela Coppola et al.,2020)

In particular, the membranes are used in periodontal and implant therapy to guide soft tissue regeneration and inhibit the rapid regrowth of skin when implanting bone which takes longer to generate in food applications, gelatin is widely used in confectionery products, jellies, yogurts, and ice creams, while in pharmaceuticals it is a primary material for capsule shells and drug delivery systems. Additionally, biomedical applications of gelatin include wound dressings, scaffolds for tissue engineering, and drug carriers (Gomez-Guillen et al.,2011).These limitations have driven the search for alternative, safe, and culturally acceptable gelatin sources. The gelatin produced via microbe-mediated extraction displays physicochemical properties comparable to commercial gelatin and can be used in a variety of fields: Biodegradable plastics, Anti-microbial coatings (e.g., for food preservation), Additives for plant growth under stress, Firmness enhancers in dairy products like yogurt, Anti-adhesion films effective against bacterial biofilms, etc. Fish-processing byproducts offer a sustainable and underutilized alternative for gelatin production. Skins, bones, and scales are particularly rich in collagen, which can be converted into gelatin with functional properties comparable to or even superior to mammalian gelatin (Muyonga et al., 2004). Gelatin derived from fish possesses several advantages: it avoids religious restrictions, demonstrates high digestibility, and often exhibits unique thermal and rheological properties depending on the species and habitat of the fish (Haug et al., 2004). Moreover, valorising fish waste into gelatin contributes to waste reduction, improved resource efficiency, and supports the principles of a circular bioeconomy.

Fish Waste as a Source of Gelatin

Among various fish-derived biomolecules, gelatin has gained exceptional attention due to its versatility and wide industrial applications. (Joy et al., 2024). Gelatin is a hydrolysed form of collagen, a structural protein abundant in fish skin, scales, and bones (Johnston-Banks, 1990). It has applications in the food industry (as gelling, thickening, stabilizing, and film-forming agent), pharmaceuticals (as capsule material and drug carrier),

cosmetics (as moisturizer and skin rejuvenator), and biomedical fields (as scaffolds for tissue engineering and wound healing) (Pu et al., 2023).

Traditionally, gelatin has been extracted from mammalian sources such as bovine hides and pigskin. However, outbreaks of zoonotic diseases like bovine spongiform encephalopathy (BSE) and cultural or religious restrictions among Muslim and Hindu communities have increased the demand for non-mammalian sources, particularly fish gelatin (Karim & Bhat, 2009). Fish gelatin is recognized as halal, kosher, and environmentally sustainable, making it an attractive alternative to mammalian gelatin (Gomez-Guillen et al., 2011)

2. Properties and Advantages of Fish Gelatin

Fish-derived gelatin exhibits distinctive physical and chemical characteristics compared to mammalian gelatin. It generally possesses lower melting and gelling temperatures, making it more suitable for cold-set food formulations (Jamilah & Harvinder, 2002). Warm-water and tropical species, including Indian major carps, have been reported to yield gelatin with gel strength comparable to commercial bovine gelatin (Gudmundsson & Hafsteinsson, 1997; Ahmad & Benjakul, 2011). Recent studies show that fish gelatin contains high proportions of glycine, proline, and hydroxyproline—amino acids responsible for gel strength and film formation (Muyonga et al., 2004; Haug et al., 2004).

Moreover, recent advancements (2018–2025) have expanded gelatine's applications beyond food gelling agents. Studies report its potential in active food packaging, antimicrobial coatings, biodegradable films, and cosmetic formulations (Kouhdasht & Panahirad, 2021). For instance, (Rocha-Pimienta et al. 2023) demonstrated that gelatin extracted from fish waste exhibits strong antimicrobial and antioxidant potential, making it suitable for biopolymer film development. Similarly, (Boronat et al. ,2022) utilized fish-waste-derived gelatin for UV-blocking biodegradable films, highlighting its functional and environmental value.

2.1 Applications of Gelatin:

- **Food Industry:** Used as a gelling, thickening, and stabilizing agent in desserts, yogurts, confectionery, marshmallows, and meat emulsions. Food and nutraceuticals: low-calorie gels, stabilizers, and protein supplements
- **Pharmaceuticals:** Employed in soft and hard capsule shells, wound dressings, and drug delivery systems.
- **Cosmetics:** Added in creams, face masks, and hair products for moisturizing and anti-aging effects.
- **Biomedical Field:** Applied in tissue engineering scaffolds, wound healing hydrogels, and regenerative medicine.
- **Packaging Industry:** Formulated into biodegradable and edible films to replace synthetic plastics.
- **Agriculture:** Used for coating seeds, controlled-release fertilizers, and biodegradable soil conditioners.



REVIEW OF LITERATURE



Development And Characterization Of Gelatin-Based Bioplastic Derived From Fish Scale Waste.

Review Of Literature

The utilization of fish-processing waste represents a sustainable approach to reducing environmental pollution while recovering valuable biomaterials. This study aimed to extract gelatin from the skin of freshwater fish and to evaluate its physical, chemical, and biochemical characteristics. The extraction was performed using acid pretreatment followed by thermal extraction under controlled conditions to obtain high-quality gelatin. The extracted product was analysed for its physical properties (colour, texture, gel strength, and viscosity) and chemical composition (moisture, ash, and protein content). Biochemical analyses (in vitro) were carried out to assess the purity and stability of the gelatin, while morphological examination provided insights into its surface structure and uniformity. The obtained results were compared with previously reported findings, particularly those of (Rabia et al., 2018), indicating that gelatin derived from freshwater fish skin possesses comparable quality to commercial mammalian gelatin. This research highlights the potential of fish skin as an eco-friendly and economically viable alternative source of industrial gelatin, supporting waste valorisation and sustainable resource utilization.

1. Fish Consumption And The Fish Industry

Fish products are an important source of protein containing 15 – 20 % of protein (Usman et al., 2022) The fish industry also supports a large workforce: over 60 million people are directly involved in fishing and aquaculture, and more than 500 million depend on related activities for their livelihood (FAO 2022).

Fish is one of the most important sources of animal protein for people all around the world. According to the Food and Agriculture Organization (FAO 2024), fish provides about 17 percent of the total animal protein intake of the global population. Over the past few decades, fish consumption has increased rapidly from around 9 kg per person per year in 1961 to more than 20 kg in 2020 (FAO 2024; OECD-FAO Agricultural Outlook 2022–2031). This rise is mainly due to the growth of aquaculture, or fish farming, which now supplies over 50 percent of all fish eaten by humans (FAO SOFIA 2022). Asia plays the biggest role in both fish production and consumption. Countries such as China, India, Indonesia, and Vietnam together

account for more than 70 percent of global fish consumption (OECD-FAO Outlook 2023).

1.1 Fishery Industry Waste

The fishery industry plays a vital role in the global food supply, yet it also generates a substantial amount of waste during processing. Fish and shellfish are harvested from a wide variety of aquatic species, but usually, only the most desirable and easily edible portions such as fillets are used for human consumption. The remaining parts, including heads, skins, bones, fins, scales, and viscera, make up a large portion of the catch and are often discarded as waste or used as low-value byproducts. These discarded portions are rich in nutrients and proteins, representing a significant underutilized resource (Wasswa et al., 2007). Globally, fish and shrimp production has been increasing steadily, reflecting the growing demand for seafood products. According to the Food and Agriculture Organization (FAO, 2002), of the estimated 131 million tonnes of fish produced in 2000, approximately 74% (97 million tonnes) was directly used for human consumption, while the remaining 26% was processed into non-food products such as fish meal and oil. As fish is a highly perishable commodity, efficient and sustainable processing methods are crucial to prevent spoilage and maximize utilization (Arvanitoyannis & Kassaveti, 2008). These byproducts, when properly utilized, not only help reduce pollution but also generate economic benefits for the industry (Shahidi, 2020). In India, the fishery sector is one of the most rapidly expanding food industries, producing millions of tonnes of seafood annually. However, it also generates a significant quantity of waste. It is estimated that the industrial fish processing sector in India produces about 3, 02,750 tonnes of waste annually (Anon., 2005). Most of this waste remains underutilized or is discarded into the environment, leading to serious ecological concerns such as odour, increased biochemical oxygen demand (BOD), and microbial contamination of water sources (Venugopal & Shahidi, 1995).

1.2 Fish Scale & Skin-

Fish scales are composed of both biological and inorganic substances. The biological portion - which comprises collagen, fat, lecithin, sclerotin, and vitamins, accounts for around 41–45% of the scale (Syandri et al., 2023).

About 38–46 % of it is inorganic portion and is made up of calcium phosphate, hydroxyapatite, and Ca, Mg, Fe, and Zn (Syandri et al., 2023).

Fish take up various elements from both the food they consume and the surrounding waters that later could be accumulated into calcified matrices such as scales and otoliths (Khawar et al., 2024; Pourang, 1995; Varol et al., 2022). Such hard structures continue to grow throughout their lives, allowing the geochemical alterations to be retained in these tissues as long-term records of metal exposure across the life span (Filipović Marijić et al., 2022). Fish skin and scales are important structural parts of the fish body that protect it and also serve as valuable raw materials for many industries. Both skin and scales are rich in collagen, a natural protein that gives strength, flexibility, and elasticity to tissues & Fish scales, which usually make up about 1–5% of a fish's total weight, (Jafari et al., Polymers 2020).

1.3 Uses of Fishery Industry Wastes.

The fishery industry produces large quantities of waste materials such as heads, bones, skins, fins, scales, and viscera, which can cause environmental contamination if not properly managed (Laufenberg et al., 2003). It is estimated that more than 50% of the total fish catch is not used directly as food, generating approximately 32 million tonnes of waste annually (Kristinsson & Rasco, 2000). To address this issue, researchers have developed various methods to convert fish waste into valuable products such as animal feed, biodiesel, pigments, and functional ingredients for food and cosmetics.

➤ Natural Pigments

Shrimp waste is a rich source of natural carotenoids, primarily astaxanthin, which are valuable natural colorants used in aquaculture and the food industry. Carotenoids are extracted from shrimp heads and shells using organic solvents (Sachindra et al., 2001). The residue after pigment extraction can still be processed for chitin and chitosan recovery, adding further value to the waste (Sachindra et al., 2006).

➤ **Food Industry and Cosmetic Applications**

Seafood waste also provides raw materials for bioactive compounds such as enzymes, gelatin, collagen, and protein hydrolysates, which have antimicrobial, antioxidant, and anti-tumour properties (Haard & Simpson, 1994). Chitosan from shrimp and crab shells is widely used in pharmaceutical and cosmetic formulations (Mandeville et al., 1992).

Shrimp head protein hydrolysates (SHPH) have been shown to improve moisture retention and reduce protein denaturation in food products (Ruttanapornvareesakul et al., 2005). Similarly, fish protein hydrolysates (FPH) can act as natural cryoprotectants to preserve fish muscle quality during frozen storage and can also be used as nutrient sources for microbial culture media (Khan et al., 2003; Martone et al., 2005).

2. Gelatin

Gelatin is a hydrocolloid widely applied in confectionery, dairy products, capsules, and cosmetic formulations due to its unique gelling, emulsifying, and stabilizing properties (Karim & Bhat, 2009). Traditionally, the majority of gelatin is produced from porcine and bovine sources through acid, alkali, or hot-water extraction of hides, bones, and skins. However, these conventional sources face several limitations. Religious restrictions in Islamic communities prohibit the consumption of pork-derived products, while Hindu traditions avoid bovine sources. Moreover, public health concerns such as bovine spongiform encephalopathy (BSE) and swine influenza have further reduced consumer acceptance of mammalian-derived gelatin (Karim & Bhat, 2009). Marine collagen and bovine collagen differ slightly in the types of collagen. As you might expect, collagen comes from fish -is found in fish skin and scales. If you eat the skin when you have a meal that includes fish, you're essentially getting extra collagen. Marine collagen supplements are those that contain collagen derived from these fish sources. They are therefore the preferred choice for pescatarians or anyone who eliminates beef from their diet (Karim & Bhat, 2009).

2.1 Beneficial Properties Of Gelatin

Gelatin is produced through the partial hydrolysis of collagen and is widely used in the food, pharmaceutical, and photographic industries. These characteristics depend on several factors including the source of collagen, pretreatment method, extraction conditions, pH, temperature, and interacting compounds during processing (Cole, 2000). Functionally, gelatin can be classified into two major groups:

- Gelling properties, including gel strength, viscosity, elasticity, and water-binding ability.
 - Surface-active properties, including emulsification, foam formation, film formation, and adhesion (Schrieber & Gareis, 2007).
1. Solubility in water.
 2. Gelling properties.
 3. Emulsifying and foaming properties

➤ Gelling Properties

One of gelatin's most important features is its thermo-reversible gelation, meaning it forms gels when cooled and melts when heated. This property gives gelatin desserts their unique "melt-in-the-mouth" texture (Glicksmann et al., 1969). Other hydrocolloids such as agar or pectin can form gels but do not replicate the elastic, clean, and smooth texture of gelatin (Baziwane & He, 2003). Gel strength depends on molecular weight lower molecular weight gelatins yield weaker gels. The α -chain (100 kDa) of collagen contributes most to gel strength, while higher molecular weight components contribute less (Cole, 2000). In confectionery applications, gelatin acts as a gelling and binding agent in gummies and wine gums. However, incompatibility with glucose syrups or the presence of proteolytic enzymes in fruits like pineapple can cause gel failure unless enzymes are deactivated by heating (Marrs et al., 1982).

2.2 Benefits Related To Human Health:

Gelatin is rich in collagen -derived proteins which help to maintain joint cartilage and bone density .It also improves skin, hair and nail health, Gelatin maintain,skin,elasticity,hydration.

Gelatin can bind with water in digestive tract, improving food movement and intestinal barrier integrity .Glycine in gelatin acts as calming neurotransmitter.

Gelatin provides wide range of health benefits , as some are mention below

1.Effect Of Fish Gelatin on Gut

2. Effect Of Fish Gelatin on Sleep

➤ **Effect Of Fish Gelatin On Gut**

Healthy Gut with Fish Gelatin being more bioavailable, the body doesn't have to work so hard to metabolism it. This is great benefit for anyone with gut issues. What's more, marine collagen contains glycine, which has been linked to a reduction in inflammation in the digestive tract; gelatin peptides from different food sources can serve as a nitrogen or carbon source for microorganisms in the intestinal tract, generating fermentation products and favourable growth mediators of gut microbiota, thus exerting a prebiotic effect. By regulating the composition of intestinal flora or metabolites, it is a reasonable means to improve certain diseases and maintain a healthy state (Baojing Ren et al.,2024)

➤ **Effect Of Fish Gelatin On Sleep**

Glycine, a non-essential amino acid naturally present in fish gelatin has been shown to positively impact sleep and help with insomnia. It may also regulate our body's core temperature which, again helps us to sleep better (Holmes, D.F.Lu,et al.,2018). Collagen (gelatin) Peptides may also be more effective than glycine alone due to its potential effects on nerve growth factors & it reduces awakenings and improved cognitive function in physically (Craig Thomas et al.2023) [European Journal of Nutrition].

2.3 Gelatin Based Edible Films

Edible films made from biopolymers act as protective barriers against moisture, oxygen, and gases, extending food shelf life (McHugh, 2000). Among proteins, gelatin and collagen are preferred due to their biodegradability, film-forming ability, and abundance (Bigi et al., 2002; Gennadios et al., 1997) Gelatin films have been applied to meat and poultry to prevent oxidation, colour loss, and microbial growth (Villegas et al., 1999; Cutter & Miller, 2004). They also serve as

antioxidant carriers, helping preserve flavour and aroma (Gennadios et al., 1997). However, gelatin alone forms weak films and often requires drying or reinforcement. Collagen-based coatings are widely used in the meat industry for ham, roasts, and sausages, improving juiciness, smoke permeability, and texture (Cutter & Summer, 2002).

2.4 Plasticizers In Edible Films

Plasticizers such as glycerol, sorbitol, and propylene glycol are added to improve flexibility and reduce brittleness by weakening intermolecular bonds (Sobral et al., 2002).

They lower the glass transition temperature, increasing film elasticity but decreasing moisture and oxygen barrier capacity (Bergo & Sobral, 2007). Studies showed that plasticizers alter gelatine's mechanical properties without causing recrystallization, making films more stretchable and humidity-sensitive (Lukasik & Ludescher, 2006).

2.5 Fish Gelatin-Based Products

The largest food application of gelatin is in water gel desserts, known for their “melt-in-the-mouth” texture (Zhou & Regenstein, 2007). Fish gelatin serves as an excellent Halal and Kosher alternative to pork or bovine gelatin and produces smoother flavour release with less off-odor (Choi & Regenstein, 2000).

2.6 Microorganisms Involved In Gelatin Extraction

Microorganisms play a crucial role in enzymatic extraction of gelatin from fish by products. Common microbes –*Bacillus subtilis*, *Bacillus licheniformis*, *Aspergillus oryzae*, *Rhizopus sp.*, *Streptomyces sp.* Which produce extracellular proteases capable of breaking down collagen fibers in fish skin, scales, and bones. Gomez-Guilien et al., 2011). Produce proteolytic enzymes that selectively hydrolyze collagen into gelatin peptides. Microbial enzymes allow controlled hydrolysis, preserving functional properties and reducing chemical waste (Benjakul & Morrissey, 2005) These microbial enzymes, such as alcalase, trypsin, and papain-like proteases.

3. Alternative Sources: Fish Scales And Other By-Products

Fish scales, a by-product of fish processing, have emerged as a promising alternative source for gelatin production. (Shahidi & Synowiecki, 1991) .Other alternative sources of gelatin have also been explored but each varies in collagen content, yield, and functional properties (Benjakul & Morrissey, 2005).

Including:

- Fish bones -Fish bones contain collagen but require careful demineralization prior to extraction.
- Fins – while fins provide a smaller but valuable amount of collagen.
- Jellyfish -Jellyfish gelatin, increasingly studied in Asia, offers unique thermal and rheological Properties, though its large-scale production is still limited. (Gomez-Guillen et al., 2011)

Using these alternative sources not only diversifies raw material supply but also contributes to sustainable fish waste management, reducing environmental impact and maximizing resource utilization. Fish scales: Rich in collagen type I; gelatin is lighter in colour and transparent (Gómez-Guillén et al., 2011).

4. Economic Impact Of Fish Gelatin Production:

- Along the production the production of gelatin from fish waste offers significant economic benefits by converting underutilized by-products, such as skins, scales, and bones, into a valuable coproduction.
- These by-products, often discarded and potentially costly to manage, can instead generate additional income for fisheries and processing facilities. (Gomez-Guillen et al., 2011).
- Fish gelatin is in increasing demand across the food, pharmaceutical, and cosmetic industries, particularly due to the growing market for halal, kosher, and sustainable products.
- While methods like enzymatic extraction may involve higher initial costs for enzymes and precise monitoring, the resulting higher yield and superior quality gelatin can offset these expenses. (Benjakul & Morrissey et Al., 2005).

Moreover, this approach reduces waste management costs and creates employment opportunities and distribution chain, making fish gelatin a profitable and sustainable economic venture (Benjakul & Morrissey et al., 2005).

5. Importance Of Gelatin Produced By Using Fish Waste:

- Combines sustainability, high functional quality, and economic feasibility.
- Microbial enzymes selectively hydrolyse collagen, preserving gel strength, viscosity, and clarity (Gomez-Guillen et al., 2011).
- Reduces chemical waste and energy consumption compared to chemical/thermal methods (Benjakul & Morrissey, 2005).
- Supports waste valorization, promotes a circular economy, and expands applications for halal/kosher and dietary-restricted products (Shahidi & Synowiecki, 1991).
- Microbe-mediated gelatin extraction from fish waste is increasingly recognized as one of the most efficient, sustainable, and high-quality methods for producing gelatin. Unlike chemical or purely thermal methods, microbial enzymes provide selective hydrolysis of collagen, preserving the functional properties of gelatin, such as gel strength, viscosity, and clarity (Gomez-Guillen et al., 2011).
- The process is eco-friendly, generating minimal chemical waste and requiring lower energy compared to thermal extraction (Benjakul & Morrissey, 2005). Fish waste itself is abundant, inexpensive, and underutilized, making it an ideal raw material that supports waste valorization and a circular economy. Furthermore, fish gelatin obtained through microbial methods is suitable for halal, kosher, and dietary-restricted applications, which expands its market potential (Shahidi & Synowiecki, 1991).

i. Food Applications

Gelatin is extensively used in the food industry as a gelling agent, stabilizer, emulsifier, and thickener (Cole, 2000). Common applications include desserts, confectionery, dairy products, meat coatings, and beverages. It functions as a clarifying agent in fruit juices, wine, and beer (Igoe, 1983), and as a thickener in yogurt (0.3–0.5%), ham coatings (2–3%), and desserts (8–10% of dry weight). Gelatin is also found in instant soups, gravies, sauces, and edible films for confectionery (McCormick, 1987).

In low-fat spreads, gelatin serves as a fat substitute and texture stabilizer, often combined with pectin to improve spread ability and mouthfeel (Cheng et al., 2007). The largest single use of gelatin in food products is in water-based gel desserts, where its thermo-reversible gelation provides the unique “melt-in-the-mouth” quality (Zhou & Regenstein, 2007). Fish-derived gelatin, being free from religious and health restrictions associated with mammalian sources, offers an alternative for consumers seeking Halal, Kosher, or BSE-free options (Choi & Regenstein, 2000).

ii. Pharmaceutical Applications

Gelatin plays an essential role in the pharmaceutical and nutraceutical industries, primarily in the production of hard and soft capsules, tablets, and microencapsulated powders (Schrieber & Gareis, 2007). High-bloom gelatin (200–260 Bloom) is used in hard capsule manufacture, while fish gelatin, when cross-linked using transglutaminase, can also be used for capsule films with improved strength (Park et al., 2007).

Additionally, fish gelatin with Bloom values above 100 has been applied in drug tablet formulations (Hansen et al., 2002). Gelatin is also used to microencapsulate oil-soluble vitamins, forming stable, freely flowing powders through spray-drying and cross-linking (Cole, 2000). In medical applications, special types of gelatin serve as blood plasma expanders, biological adhesives, and absorbable surgical sponges, which can be impregnated with antibiotics for wound healing .

Furthermore, gelatin hydrolysate supports joint health by mimicking the amino acid composition of cartilage and has shown positive effects in reducing arthritis pain and bone loss when consumed at about 10 g/day .

iii. Photographic And Imaging Applications

Photographic-grade gelatin is used to bind light-sensitive silver halide crystals to film and photo paper. It enables the formation of multiple thin, uniform layers on carrier materials and maintains stability during image development .Gelatine’s swelling properties and gelling behaviour facilitate the coating and processing of photographic emulsions, ensuring image sharpness and contrast. In colour photography, gelatin stabilizes dye and

coupler emulsions and enhances print brightness and tone (Schrieber & Gareis, 2007).

Additionally, innovative gelatin types such as Image are designed for inkjet photo paper and film, producing realistic, high-gloss prints. Fish gelatin, with its low gelling temperature, is particularly valuable for light-sensitive coatings and silver halide precipitation, as it allows processing at lower temperatures than mammalian gelatines (Schrieber & Gareis, 2007).

iv. Industrial And Other Applications

In non-food and non-pharmaceutical industries, gelatin serves as a binding agent, adhesive, and microencapsulation medium. Industrial gelatin is used in carbonless paper, cosmetics, paint formulations, and microencapsulation of pigments and fragrances. It also finds use in adhesives and glues, leveraging its strong cohesive and film-forming abilities while remaining biodegradable and environmentally friendly (Schrieber & Gareis, 2007).

6..Advantages Of Fish Waste Gelatin:

- Gelatin derived from fish waste offers several important benefits. First, it provides a sustainable use of fish by-products such as skin, scales, and bones, reducing environmental pollution and promoting a circular economy (Gomez-Guillen et al., 2011).
- It is suitable for halal, kosher, and pescatarian diets, expanding its application in culturally and religiously sensitive markets (Benjakul & Morrissey, 2005).
- Additionally, enzymatic extraction methods allow production of gelatin with controlled molecular weight, improved functional properties, and reduced chemical waste, making the process eco-friendly (Gomez-Guillen et al., 2011).
- Enzymatic/microbial extraction allows controlled functional properties and eco-friendly production (Gomez-Guillin et al., 2011).
- Fish gelatin is often lighter in colour and more transparent compared to mammalian gelatin, making it desirable for high-quality food, beverage, and

cosmetic products. Lighter colour and higher transparency, desirable for food, cosmetics, and pharmaceuticals. (Shahidi & Synowiecki, 1991).

6.1. Drawbacks

Despite these advantages, fish waste gelatin also has some constraints e.g -Lower gel strength and melting temperature compared to mammalian gelatin. generally lower than bovine or porcine gelatin, which can limit applications in products requiring firm gels (Benjakul & Morrissey, 2005).

- Lower yield from certain by-products such as scales and bones compared to skin (Shahidi & Synowiecki, 1991).
- Enzymatic extraction requires enzyme cost and precise control of conditions (Gomez-Guillen et al., 2011). Additionally, enzymatic extraction, while environmentally friendly, can be costly due to enzyme procurement and requires careful control of pH, temperature, and hydrolysis time to prevent over-digestion and loss of functionality.

7. Plastic based products

In present times, plastic pollution has become one of the most serious environmental problems worldwide due to the excessive use and improper disposal of plastic materials in daily life. Plastics are non-biodegradable, meaning they do not decompose easily and can remain in the environment for hundreds of years, leading to their continuous accumulation on land and in water bodies. Rapid urbanization, increased consumption, and the widespread use of single-use plastics such as bags, bottles, and packaging materials have worsened the situation. As a result, large amounts of plastic waste end up in rivers, lakes, and oceans, causing severe harm to marine life, as animals often ingest plastic or become entangled in it. Over time, plastics break down into microplastics, which contaminate soil and water and enter the food chain, ultimately affecting human health. Additionally, plastic waste clogs drainage systems, leading to waterlogging and flooding, especially in urban areas. The burning of plastic releases toxic gases, contributing to air pollution and respiratory diseases.. Despite recycling efforts, a large portion of plastic waste is not effectively managed, making it a persistent global challenge. Therefore, reducing plastic use, promoting alternatives like bioplastics, improving waste management systems, and

increasing public awareness are essential steps to tackle the growing plastic pollution problem and ensure a healthier and more sustainable environment.

8. Bioplastic-

Bioplastics made from fish scale waste are an innovative and sustainable solution that addresses both plastic pollution and fish processing waste management. Fish scales, which are usually discarded as waste, are rich in proteins like collagen and gelatin, making them a valuable raw material for producing biodegradable bioplastics. The use of fish scale waste helps in converting low-value waste into high-value eco-friendly products, thereby reducing environmental pollution and supporting waste-to-wealth approaches. These bioplastics are biodegradable and can naturally decompose into harmless substances, unlike conventional plastics that persist in the environment for hundreds of years. Additionally, producing bioplastics from fish scales reduces dependence on petroleum-based resources and lowers carbon emissions, contributing to a cleaner environment. Such bioplastics are especially useful in packaging, agricultural films, and biomedical applications due to their biocompatibility, non-toxicity, and film-forming properties. Moreover, this approach supports sustainable fisheries and reduces the burden on landfills and water bodies where fish waste is often dumped, causing foul odor and pollution. Although there are challenges such as cost, large-scale production, and durability, the development and use of fish scale-based bioplastics represent an important step toward sustainable development, circular economy practices, and effective management of both plastic and organic waste.

9. Research Area For Future Studies:

However, most of these extraction techniques rely on heavily on chemical pretreatment and thermal hydrolysis, which generate effluents, degrade gelatin quality, and increase production cost. Conventional gelatin extraction faces several problems as it involves multiple steps processes using acid (Type A) or alkali (Type B) pretreatment, followed by prolonged hot -water extraction. (Norziah et al., 2009).

While effective, these methods pose several drawbacks:

1. extensive chemical usage leading to environmental hazards
2. long processing times
3. inconsistency in gelatin quality
4. denaturation of bioactive compounds

These challenges highlight the urgent need for alternative green extraction technologies that are both efficient and environmentally sustainable. Edible coatings and films based on gelatin can be prepared, leading to the development of new strategies for the cost-effective and recyclable packaging of food (Merina et al., 2017). The functional properties of these films can be enhanced by adding different substances like cross-linkers and plasticizers. Bioactivity testing showed that obtained collagen peptides had antioxidant activity, reducing free radicals at 10 mg/mL, higher than those of hyaluronic acid, an ideal material in cosmetics. The obtained hydrolysates (in which collagen peptides accounted for approximately 95%) also had promoting cell-proliferation effects on human dermal fibroblasts and showed no toxicity (Daniela Coppola et al., 2020).



OBJECTIVE

Objectives

1. Collection of fish scale waste from local fish market areas and its Cleaning and pretreatment
2. Isolation and characterization of microorganisms from soil and fish scale samples.
3. Screening of bacterial isolates for protein degradation ability using skim milk agar.
4. Extraction of gelatin from fish scales using alkaline, acid, and thermal treatment methods.
5. Evaluation of the physical characteristics of the extracted gelatin, including colour, texture, solubility, and gel formation.
6. Preparation of biodegradable bioplastic films using fish scale-derived gelatin and glycerol as a plasticizer.



MATERIAL AND METHODS



Development And Characterization Of Gelatin-Based Bioplastic Derived From Fish Scale Waste.

❖ MATERIAL AND METHODS

❖ Collection Of Samples

The present study focused on isolation of those organisms which have fish waste degrading ability. So we have selected fish waste dumping site soil and fish scale as source of isolation.

Two sample was collected-

1. soil sample
2. fish scale sample

1.Collection Of Soil Sample-

The study focused on collection of soil sample from fish waste dumping sites so chances of getting organisms directly adapted to fish waste are very high. soil sample was collected from various location Like Peth vadgaon, Kuditre, Kolhapur, N.P.fish market dumping area Kolhapur, Sangali fish market, koge dam, uachgaon, friendship fish market bavda, Shirol, shigaon, etc.

Table No 1 – Soil sample location

| Sr.no | Sample | Sample location |
|-------|--------|-----------------|
| 1 | A | Peth vadgaon |
| 2 | Sk | Kuditre |
| 3 | K | Kolhapur |
| 4 | N.P | N.P.fish market |
| 5 | S | Sangali |

❖ Physical Analysis:

Collected soil sample was analysed for further study to determine various characteristics of soil which includes,

1) Texture:

-Collected soil sample identified by moisture of soil and checked for grittiness (sand), smoothness (silt), or stickiness (clay).

2) Colour:

- Soil colour is indicator of its composition and organic matter content.
- Dark soil is typically rich in organic matter & light color indicates sandy texture & low organic matter, while red soil is found to be rich in iron oxide.

3) PH

Soil pH determination is the process of measuring the acidity or alkalinity of soil, which influences nutrient availability and plant growth. It also affects the growth and the activity of microorganisms.

❖ Isolation of fish scale degrading organism from soil sample-

For isolation we used sterile nutrient agar medium plates because it is non-selective, general-purpose medium that provides essential nutrients (peptone, beef extract) to support the wide variety of non-fastidious heterotrophic microorganisms found in soil, allowing them to form distinct, isolated colonies for screening.

Table No 2- Composition of nutrient agar medium

| Component | Quantity in gm |
|---------------------------|----------------|
| Peptone | 0.5 |
| Beef extract/meat extract | 0.3 |
| Sodium chloride | 0.5 |
| Agar agar | 1.5 |
| Distilled water | 100ml |
| pH | 7 |

❖ Preparation of media-

100 ml of distilled water was taken and media was prepared using the required components and sterilized by autoclaving. Then after sterilization, media was poured in petri dish and used for further isolations.

❖ Preparation of soil sample

1gm of soil sample was taken then it was serially diluted with sterile distilled water blanks from 10^{-1} to 10^{-10} .

❖ Inoculation and incubation

1. To isolate bacteria from sample, sterile nutrient agar plates were prepared
2. On each plate respective dilutions of soil samples [dilution 10^{-3} , 10^{-4} , 10^{-5}] were spread by using spread plate technique
3. Incubation was done at 37°C for 24-48 hours. after incubation colonies was observed.
4. We isolated number of colonies from different dilutions of soil sample. we selected some potent colonies for further morphological character and biochemical characters.

2. Collection Of Fish Scale Sample

Fish scales are used to study degrading organisms because they are highly biodegradable, nutrient-rich substrates composed mainly of type I collagen and hydroxyapatite. They act as a model material to isolate, identify, and cultivate microorganisms (specifically bacteria and fungi) that possess the metabolic capacity to break down complex organic protein-based wastes, thus aiding in waste management and identifying new proteolytic enzymes. For the isolation fish scale sample collected from various Location Like Peth, Vdgaon, Kolhapur, Kuditre, N.P.fish market Kolhapur, Koge dam etc. was used

Table No 3 - Fish scale sample and their location

| Sr.no | Sample | Location |
|-------|--------|-----------------|
| 1. | A | Peth vadgaon |
| 2. | Fk | Kuditre |
| 3. | K | Kolhapur |
| 4. | N. P | N.P.fish market |
| 5. | KD | Koge dam |

❖ Isolation Of Fish Scale Sample Cultures

For the isolation Nutrient agar is used to cultivate a broad range of non-fastidious heterotrophic bacteria present on fish scales. Skim milk agar acts as a

differential medium to detect proteolytic bacteria—those producing enzymes that hydrolyze casein—indicated by clear zones around colonies, as it mimics protein-rich environmental conditions.

❖ Preparation Of Media-

100 ml of distilled water was taken and media was prepared using the required components and sterilized by autoclaving. then after sterilization, media was poured in sterile petri plates and used for further studies.

Table No 4- Nutrient agar composition

| Components | Quantity (gm) |
|---------------------------|---------------|
| Peptone | 0.5 |
| Beef extract/meat extract | 0.3 |
| Sodium chloride | 0.5 |
| Agar agar | 1.5 |
| Distilled water | 100ml |
| pH | 7 |

Table No 5 -Skim milk agar composition

| Components | Quantity (gm) |
|-----------------|---------------|
| Milk | 10ml |
| Yeast extract | 0.25 |
| Dextrose | 0.01gm |
| Agar | 1.5 |
| Distilled water | 100 ml |
| pH | 7 |

❖ Preparation of fish scale sample dilution-

1 gm of fish scale sample from each sample was measured and added into 1st sterile distilled water (10ml) tube and labelled as 10^{-1} then it was serially diluted from 10^{-1} to 10^{-10} by using dilution blank.

❖ **Inoculation & incubation:**

For the isolation of fish scale degrading bacteria, sterile nutrient agar & skim milk agar plate were prepared.

- ▶ On each plate respective dilutions of respective samples [dilution 10^{-3} , 10^{-4} , 10^{-5}] were spread by using spread plate technique and plates were incubated at 37°C for 24-48 hours.
- ▶ After incubation we have observed the white & yellow colored, mucoid colonies on the nutrient agar plates.

3.Selection of potent organisms

Selection of potent organisms is important to screen selective isolates from number of isolates. We have isolated around 30 organisms from fish waste dumped soil and scale samples. On the basis of proteolytic zone observed on Skim milk agar plates, we have selected 10 potent organisms labelled as T1-T10. These organisms were further studied for their fish scale degradation ability.

4. Fish scale degradation study

❖ **Preparation of media:**

The isolated organisms were further screened for their fish scale waste degradation ability to select most potent organism for further study.

For isolation we have used minimal media because Minimal Media (MM) contains only fish scale as a source of nutrient that will help to isolate scale degrading organism from sample. This minimal media helps to identify the capacity of microorganisms to degrade collagen specifically, as it lacks easily metabolizable nutrient sources.

Table No 6-Composition of minimal media-

| Component | Quantity in gm |
|--------------------------|-----------------------|
| Glucose | 0.2gm |
| NH_4CL | 0.1gm |
| KH_2PO_4 | 0.3gm |
| K_2HPO_4 | 0.6gm |
| NaCl | 0.25gm |

| | |
|-------------------|--------|
| MgSO ₄ | 0.12gm |
| Distilled water | 100ml |
| pH | 7 |

❖ Preparation of media

- Minimal media was prepared and 1 gm fish scale was added in it and sterilized by autoclaving.
- From screening 10 potent cultures were selected and each bacterial culture was inoculated separately into flasks containing minimal media and fish scale.
- The inoculated flasks were incubated at 37°C for 5 days under shaking condition to allow bacterial growth. After each 24 hours fish degradation rate was analyzed by using fish scale cultured medium for further protein estimation study.

5. Protein estimation

Protein estimation measures the concentration of total protein in a sample, analyzed using spectrophotometry to measure color intensity which is directly proportional to protein concentration

❖ Procedure:

For the study of protein estimation, we used 10 isolated cultures

1. After every 24 hours the 1ml of cultured broth were transferred into centrifuge tubes under aseptic condition.
2. The samples were centrifuged at 5000 rpm for 10 minutes.
3. This step separated the bacterial cells (pellet) from the supernatant which was used for estimation of protein by biuret method
4. testing cycle were repeated for every 24 hours for a week to estimate amount of protein.

❖ Estimation of protein by biuret method-

1. Biuret reagent preparation

About 80 mL distilled water was taken and mixed with 10 g sodium hydroxide (NaOH) and dissolved completely.

Then 2–3 g sodium potassium tartrate and 1 g copper(II) sulfate (CuSO₄) added with stirring.

1. Preparation of casein stock solution

- Casein stock solution was prepared (1000 ug/ml).

2. Preparation of standard solutions

- Ten test tubes were labelled S1–S10.

- Different volumes of casein stock solution were added to each tube and the volume was adjusted to 1 ml with distilled water to obtain concentrations ranging from 100-1000 ug/ml.

3. Preparation of blank

-A blank solution was prepared by taking 1 ml of distilled water in a test tube and 4 ml of Biuret reagent, followed by mixing.

4. Addition of biuret reagent

-4 ml of Biuret reagent was added to each standard solution and mixed properly.

5. Preparation of test sample & incubation

- 1 ml of culture supernatant was taken in a test tube, and 4 ml of Biuret reagent was added and mixed well and then All tubes were incubated for 30 minutes at room temperature for colour development.

6. Measurement of absorbance

- The optical density (OD) was measured at 540 nm using a spectrophotometer, using the blank for calibration.

7. Standard curve Preparation

- A standard curve was plotted with protein concentration (ug/ml) on the X-axis and optical density (OD) on the Y-axis to determine the protein concentration of the sample.

6. Gelatin Extraction From Fish Scale:

Fish scale waste was used to extract gelatin from it. The extracted gelatin was characterised by several tests. The detailed protocol of gelatin extraction is followed to extract maximum quantity of gelatin from waste. Following protocol was used for extraction,

Fish scale waste was collected



Defatting done by soaking of 10gm scale in 20% ethanol for 1-2 hours (1:10 ratio) and repeated water wash was given.



Demineralization done by suspending the scale in 0.5 M HCL for 24 hours at room temperature (1:10 ratio) and then repeated water wash was given.



Scale was soaked in 0.1 M acetic acid for 24 hours at 4 °C and then repeated water wash was given.



For extraction, fish scale +100 ml distilled water was taken in a flask and it was placed in hot water bath (at 50 -70°C) for 3-4 hours with gentle stirring.



Then the mixer was centrifuged at 6000 to 7000 rpm for 30 minutes



Then concentrated solution was poured in shallow tray and allowed to cooled at 4°C until it turn to gel form



After gel formation it was cut into small gel strips.



Gelatin strips are ready to use.

Flowchart 1: Gelatin Extraction From Fish Scale

7. Tests For Physical Evaluation Gelatin:

1. Gelatin yield -

The 10 gm of fish scale was taken (W1) and then weight of gel was taken (W2). the yield of gel was calculated by given formula-

$$\text{Gel yield (\%)} = W2/W1 \times 100$$

2. PH determination-

The 5.9 gm gelatin was taken and 4.5 ml distilled water added in it and solution was mixed and then pH was checked.

3. Solubility test.

Solubility test was carried by taking 1 gm gelatin gel in 10 ml cold water and observations were noted down

4. Odor test

mild odor indicating good purification.

5. Transparency test

Transparency test was performed by Placing gel against printed text.

8. PREPARATION OF BIOPLASTIC FILM:

Furthermore, bioplastic was prepared by using fish scale waste. The detailed protocol of same is as follows,

10 gm fish scale was taken in a flask

↓

100 ml of 0.1 N NaOH solution was added in flask and incubated for 24 hrs at R.T.

↓

treated scale was added in 100 ml D/W.

↓

The solution was heated at 60 to 70°C for 3 to 4 hrs until the solution look thick yellow.

↓

10 ml glycerol was added in a filtered solution and the solution was heated.

↓

Then warm liquid was poured on clean glass or nonstick sheet and was dried for 24-72hrs

↓

The prepared film was removed and our biodegradable bioplastic film is ready.

Flowchart 2: Preparation Of Bioplastic Film from fish waste

9. Tests For Physical Evaluation Of Bioplastic

1. Water Absorption Test (Hydrophilicity Test)

The bioplastic sheet was cut into a small square (2 cm × 2 cm).

Weight the dry sample recorded W1. Then sample was put in a beaker with water and kept it for 24 hours at room temperature. Weigh the wet sample and record as W2.

Calculation Water absorption (%) = $\frac{W1 - W2}{W1} \times 100$

W1

2. Solubility Test

The Bioplastic sheet was cut in 2cm small square and weight was recorded and Placed it in 50 ml water for 24 hours. After 24 hours the weight of sample again taken and formula as following were used.

Solubility (%) = $\frac{W1 - W2}{W1} \times 100$

W1 – initial weight of sample,

W2 – final weight of sample

3. Biodegradability (Soil Burial Test)

A small piece of bioplastic was taken and Weigh was measured(W1) then it was burred 5cm deep in moist soil. After 7-10 days sample was removed and weight was taken again (W2).

Biodegradability = $\frac{W1 - W2}{W1} \times 100$

W1

W1 – initial weight of sample,

W2 – final weight of sample

4. Heat Resistance Test

A small piece of bioplastic was taken and placed in hot water.it was kept for 10 min. after this sheet was observed for shape change ,Softening, Dissolving etc.

5. Transparency Test

Plastic sheet was placed over printed text and Visibility was observed

Clear visibility = transparent plastic.

6. Burning Test

A small piece of bioplastic was burned using a flame.



RESULTS

➤ **Properties of Soil samples obtained from fish scale waste dumping areas**

After collection of soil sample, it is necessary to analyse collected soil for further studied we have determined various characteristics of soil for example texture, colour, and Temperature. The results are shown in following table 1.

Table No 7 - Properties Of Collected Soil Sample

| Sr. No | Location | Texture | Colour | Temperature | pH |
|--------|-----------------|---------|------------|---------------|-----|
| 1 | Pethvadgaon | Dry | Brown | Warm | 6.2 |
| 2 | Kuditre | Moist | Dark Brown | Moderate | 6.5 |
| 3 | Kolhapur | Moist | Brown | Slightly Warm | 6.4 |
| 4 | N.P Fish Market | Dry | Brown | Warm | 6.6 |
| 5 | Sangali | Moist | Brown | Moderate | 6.3 |

➤ **Isolation of Microorganisms obtained from fish scale waste dumping soil**

The Soil sample was serially diluted and plated on nutrient agar medium. After incubation, different bacterial growth were observed (Fig. 1). The Colonies showed variations in shape, size, colour, margin elevation and texture.

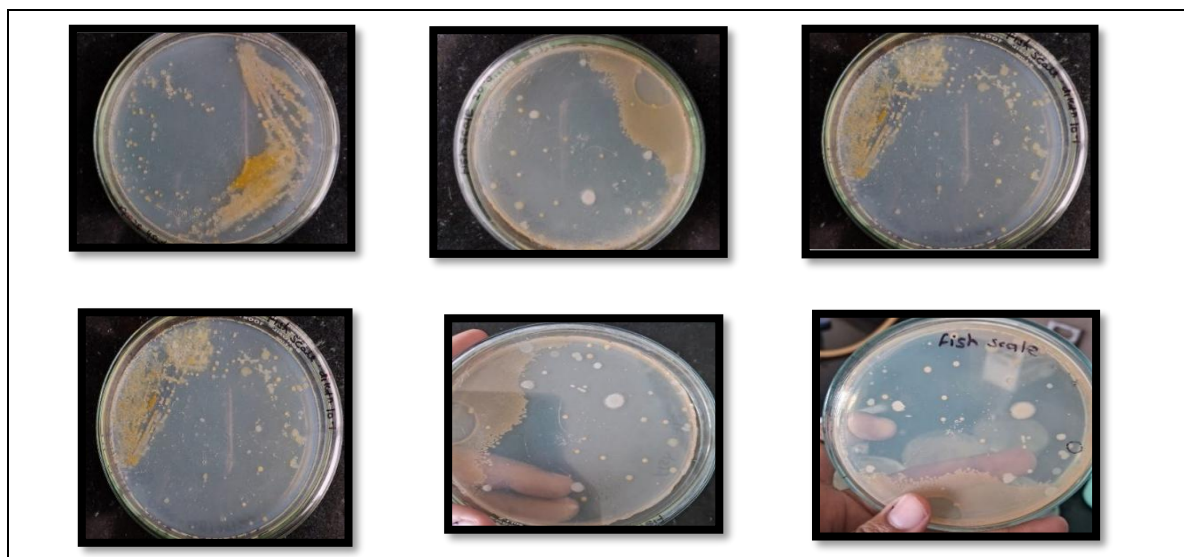


Fig No:1 Microorganisms Isolated from Fish Scale Dump Sites

➤ Colony Characteristics

Colony Characteristics of well isolated colony obtained on sterile solidified nutrient agar Plate after incubation at 37°C for 24 hours are as follows.

Colony Characteristics of isolated organism A1

| Size(mm) | Shape | Colour | Margin |
|----------|-----------|-------------|---------|
| 1.5 | Circular | Yellow | Entire |
| surface | Elevation | Consistency | Opacity |
| Smooth | Convex | Moist | Opaque |

Colony Characteristics of isolated organism A2

| Size(mm) | Shape | Colour | Margin |
|----------|-----------|-------------|---------|
| 1 | Circular | Pale yellow | Entire |
| Surface | Elevation | Consistency | Opacity |
| Smooth | Convex | Moist | Opaque |

Colony Characteristics of isolated organism A3

| Size(mm) | Shape | Colour | Margin |
|----------|------------|-------------|---------|
| 1 | Circular | White | Entire |
| surface | Elevation | Consistency | Opacity |
| Smooth | Low Convex | Moist | Opaque |

Colony Characteristics of isolated organism A4

| Size(mm) | Shape | Colour | Margin |
|----------|------------|-------------|---------|
| 1 | Circular | White | Entire |
| surface | Elevation | Consistency | Opacity |
| Smooth | Low Convex | Moist | Opaque |

Colony Characteristics of isolated organism A5

| Size(mm) | Shape | Colour | Margin |
|----------|------------|-------------|---------|
| 1 | Circular | White | Entire |
| surface | Elevation | Consistency | Opacity |
| Smooth | Low Convex | Moist | Opaque |

Colony Characteristics of isolated organism B1

| Size(mm) | Shape | Colour | Margin |
|----------|-----------|-------------|---------|
| 1.5 | Circular | Yellow | Entire |
| surface | Elevation | Consistency | Opacity |
| Smooth | Convex | Moist | Opaque |

Colony Characteristics of isolated organism B2

| Size(mm) | Shape | Colour | Margin |
|----------|------------|-------------|-------------|
| 1 | Circular | Pale Yellow | Entire |
| surface | Elevation | Consistency | Opacity |
| Smooth | Low convex | Sticky | Transparent |

Colony Characteristics of isolated organism B3

| Size(mm) | Shape | Colour | Margin |
|----------|-----------|-------------|---------|
| 1 | Irregular | Orange | Entire |
| surface | Elevation | Consistency | Opacity |
| Rough | Concave | Moist | Opaque |

Colony Characteristics of isolated organism B4

| Size(mm) | Shape | Colour | Margin |
|----------|-----------|-------------|-------------|
| 1 | Circular | Yellow | Wavy |
| surface | Elevation | Consistency | Opacity |
| Smooth | Concave | Sticky | Transparent |

Colony Characteristics of isolated organism B5

| Size(mm) | Shape | Colour | Margin |
|----------|-----------|-------------|---------|
| 2 | Circular | White | Wavy |
| surface | Elevation | Consistency | Opacity |
| Smooth | Convex | Moist | Opaque |

Colony Characteristics of isolated organism B6

| Size(mm) | Shape | Colour | Margin |
|----------|-----------|-------------|---------|
| 1.5 | Circular | Yellow | Entire |
| surface | Elevation | Consistency | Opacity |
| Smooth | Concave | Moist | Opaque |

Colony Characteristics of isolated organism C1

| Size(mm) | Shape | Colour | Margin |
|----------|-----------|-------------|-------------|
| 1 | Circular | Yellow | Entire |
| Surface | Elevation | Consistency | Opacity |
| Rough | Convex | Moist | Transparent |

Colony Characteristics of isolated organism C2

| Size(mm) | Shape | Colour | Margin |
|----------|------------|-------------|-----------|
| 1.5 | Circular | Pale Yellow | Irregular |
| Surface | Elevation | Consistency | Opacity |
| Smooth | Low convex | Moist | Opaque |

Colony Characteristics of isolated organism C3

| Size(mm) | Shape | Colour | Margin |
|----------|------------|-------------|-------------|
| 1 | Circular | Yellow | Entire |
| Surface | Elevation | Consistency | Opacity |
| Smooth | Low Convex | Sticky | Transparent |

Colony Characteristics of isolated organism C4

| Size(mm) | Shape | Colour | Margin |
|----------|-----------|-------------|---------|
| 1.5 | Circular | White | Entire |
| Surface | Elevation | Consistency | Opacity |
| Rough | Concave | Moist | Opaque |

Colony Characteristics of isolated organism C5

| Size(mm) | Shape | Colour | Margin |
|----------|-----------|-------------|---------|
| 2 | Irregular | Yellow | Entire |
| Surface | Elevation | Consistency | Opacity |
| Smooth | Convex | Moist | Opaque |

Colony Characteristics of isolated organism C6

| Size(mm) | Shape | Colour | Margin |
|----------|------------|-------------|---------|
| 2 | Circular | White | Entire |
| Surface | Elevation | Consistency | Opacity |
| Smooth | Low Convex | Moist | Opaque |

➤ Gram Nature & Motility:

All the isolated organism from soil sample was found to be gram positive most of the isolates showed cocci while few exhibited short rod-shaped morphologies (Table no. 8).

Table No 8: Gram nature and shape of organisms

| Organism | Gram Nature | Shape and arrangement of Organism |
|----------|---------------|-----------------------------------|
| A1 | Gram Positive | Cocci and singly arranged |
| A2 | Gram Positive | Cocci and Clustered arranged |
| A3 | Gram Positive | Short Rods singly arranged |
| A4 | Gram Negative | Cocci Clustered arranged |
| A5 | Gram Positive | Cocci singly arranged |
| B1 | Gram Positive | Short Rods arranged in bunch |
| B2 | Gram Positive | Cocci singly arranged |
| B3 | Gram Positive | Short rods singly arranged |
| B4 | Gram Positive | Cocci singly arranged |
| B5 | Gram Negative | Cocci Clustered arranged |
| B6 | Gram Positive | Cocci singly arranged |
| C1 | Gram Positive | Short Rods singly arranged |
| C2 | Gram Positive | Cocci singly arranged |
| C3 | Gram Positive | Cocci Clustered arranged |
| C4 | Gram Positive | Cocci Clustered arranged |
| C5 | Gram Positive | Short Rods singly arranged |
| C6 | Gram Positive | Cocci Clustered arranged |

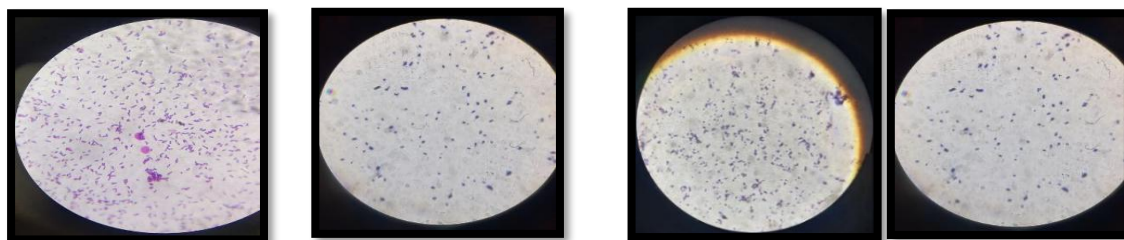


Fig No3: Gram Staining of Organism

➤ Motility

Most isolates were motile, while few are non-motility(.Table no 9)

Table No 9: Motility of organism

| Organism | Motility |
|-----------------|---------------------|
| A1 | Motile Organism |
| A2 | Motile Organism |
| A3 | Motile Organism |
| A4 | Motile Organism |
| A5 | Non motile Organism |
| B1 | Motile Organism |
| B2 | Non motile Organism |
| B3 | Motile Organism |
| B4 | Motile Organism |
| B5 | Non motile Organism |
| B6 | Motile Organism |
| C1 | Motile Organism |
| C2 | Motile Organism |

| | |
|----|---------------------|
| C3 | Non motile Organism |
| C4 | Motile Organism |
| C5 | Motile Organism |
| C6 | Motile Organism |

❖ Collection of Fish scale Sample

➤ Location & Collection of Fish Scale Sample



Fig No.4. Fish shop



Fig No.5 Collection of fish scale



Fig No 6. Fish scale collection



Fig No.7 fish scale

➤ Isolation of fish scale degrading bacteria

The collected of fish scale sample was serially diluted and plated on nutrient agar & skim milk agar medium. After incubation different bacterial colonies were observed. The colonies showed variations in shape, size, colour, margin elevation and Consistency.

Development And Characterization Of Gelatin-Based Bioplastic Derived From Fish Scale Waste.



Fig No 8: Microorganisms isolated from fish scale

➤ Colony Characteristics

Colony Characteristics of well isolated colony obtained on sterile solidified nutrient agar plate & skim milk agar Plate after Incubation at 37°C for 24 hours are as follows

Colony Characteristics of isolated organism T1

| Size | Shape | Colour | Margin |
|---------|-----------|-------------|---------|
| 1.5 | Circular | Yellow | Entire |
| Surface | Elevation | Consistency | Opacity |
| Smooth | Convex | Moist | Opaque |

Colony Characteristics of isolated organism T2

| Size | Shape | Colour | Margin |
|---------|-----------|-------------|---------|
| 1 | Circular | Pale yellow | Entire |
| Surface | Elevation | Consistency | Opacity |
| Smooth | Convex | Moist | Opaque |

Colony Characteristics of isolated organism T3

| Size | Shape | Colour | Margin |
|---------|------------|-------------|---------|
| 1 | Circular | White | Entire |
| Surface | Elevation | Consistency | Opacity |
| Smooth | Low Convex | Moist | Opaque |

Colony Characteristics of isolated organism T4

| Size | Shape | Colour | Margin |
|---------|------------|-------------|---------|
| 1 | Circular | White | Entire |
| Surface | Elevation | Consistency | Opacity |
| Smooth | Low Convex | Moist | Opaque |

Colony Characteristics of isolated organism T5

| Size | Shape | Colour | Margin |
|---------|------------|-------------|---------|
| 1 | Circular | White | Entire |
| Surface | Elevation | Consistency | Opacity |
| Smooth | Low Convex | Moist | Opaque |

Colony Characteristics of isolated organism T6

| Size | Shape | Colour | Margin |
|---------|-----------|-------------|---------|
| 1.5 | Circular | Yellow | Entire |
| surface | Elevation | Consistency | Opacity |
| Smooth | Convex | Moist | opaque |

Colony Characteristics of isolated organism T7

| Size | Shape | Colour | Margin |
|---------|-----------|-------------|---------|
| 1 | Circular | Pale yellow | Entire |
| surface | Elevation | Consistency | Opacity |
| Smooth | Convex | Moist | Opaque |

Colony Characteristics of isolated organism T8

| Size | Shape | Colour | Margin |
|---------|------------|-------------|---------|
| 1 | Circular | Orange | Entire |
| surface | Elevation | Consistency | Opacity |
| Smooth | Low Convex | Moist | Opaque |

Colony Characteristics of isolated organism T9

| Size | Shape | Colour | Margin |
|---------|------------|-------------|---------|
| 1 | Circular | White | Entire |
| Surface | Elevation | Consistency | Opacity |
| Smooth | Low Convex | Moist | Opaque |

Colony Characteristics of isolated organism T10

| Size | Shape | Colour | Margin |
|---------|------------|-------------|---------|
| 1 | Circular | White | Entire |
| surface | Elevation | Consistency | Opacity |
| Smooth | Low Convex | Moist | Opaque |

➤ Gram Nature & Motility

Table No 10: Gram Nature of organism

| Organism | Gram Nature | Shape and arrangement Of Organism |
|----------|---------------|--------------------------------------|
| T1 | Gram Positive | Cocci arranged in clustered |
| T2 | Gram Positive | Short Rods arranged singly |
| T3 | Gram Positive | Short Rods arranged in bunch |
| T4 | Gram Positive | Cocci arranged singly |
| T5 | Gram Positive | Short Rods arranged in bunch |
| T6 | Gram Positive | Short Rods arranged in bunch |
| T7 | Gram Positive | Cocci arranged in singly |
| T8 | Gram Positive | Short Rods arranged in bunch |
| T9 | Gram Positive | Cocci arranged in cluster |
| T10 | Gram Positive | Short Rods arranged singly |

Table No 11: Motility of organism

| Organism | Motility |
|-----------------|----------------------|
| T1 | Motile Organism |
| T2 | Motile Organism |
| T3 | Motile Organism |
| T4 | Non-Motile Organism |
| T5 | Motile Organism |
| T6 | Non-Motile Organism |
| T7 | Motile Organism |
| T8 | Non-Motile Organism |
| T9 | Motile Organism |
| T10 | Non -Motile Organism |

➤ Biochemical Characteristics

Biochemical profiling was performed on the basis of the glucose, mannitol, maltose, lactose, xylose, arabinose and IMVIC -Indole test, Methyl red test, Voges Prousker test.(Table no.12&13)

Table No:12 Biochemical Tests

| Organism | Glucose | Mannitol | Maltose | Lactose | Xylose | Arabinose |
|----------|---------|----------|---------|---------|--------|-----------|
| T1 | + | + | + | - | + | + |
| T2 | + | + | + | + | + | + |
| T3 | - | + | + | + | - | + |
| T4 | + | - | - | + | + | - |
| T5 | - | + | + | - | + | + |
| T6 | + | + | + | + | + | - |
| T7 | + | - | + | - | - | + |
| T8 | + | - | + | + | + | - |
| T9 | + | + | - | + | - | + |
| T10 | + | + | + | - | + | + |

Table No 13: IMVIC Tests

| Organisms | Indole Test | Methyl Red Test | Voges Proskauer Test | Citrate Utilization Test |
|------------------|--------------------|------------------------|-----------------------------|---------------------------------|
| T1 | - | - | - | - |
| T2 | - | - | + | + |
| T3 | + | + | - | - |
| T4 | - | - | - | + |
| T5 | - | + | - | - |
| T6 | - | - | - | + |
| T7 | - | - | - | - |
| T8 | - | - | + | + |
| T9 | - | + | + | - |
| T10 | - | + | - | + |
| T11 | + | - | - | + |



Fig No 9: Indole Test

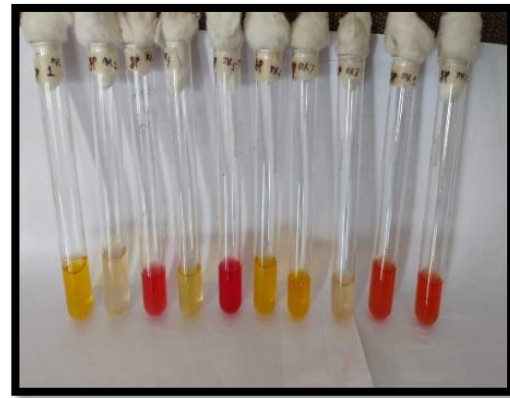


Fig No 10: Methyl red test

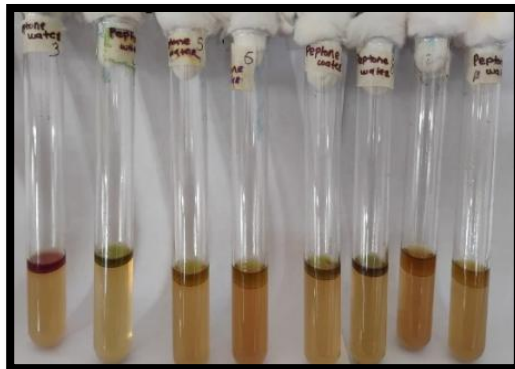


Fig No 11: Voges Proskauer Tests



Fig No 12: Citrate Utilization Test

❖ Fish Scale Degradation Study

➤ Estimation Of Protein -Preparation Of Minimal Media



Fig No 10: Bacterial Cultures of isolates Before inoculation



After Inoculation

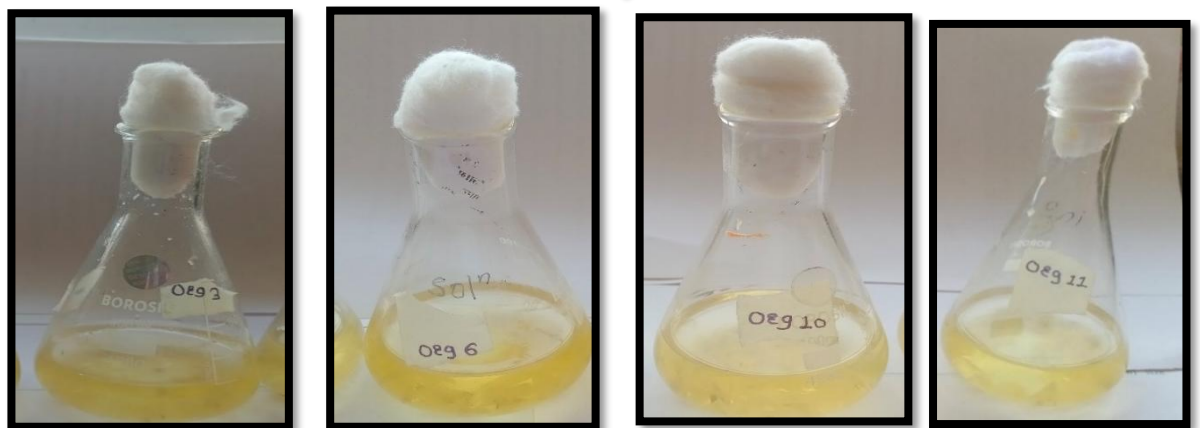


Fig No 11: Fish Scale Degraded in Bacterial Culture

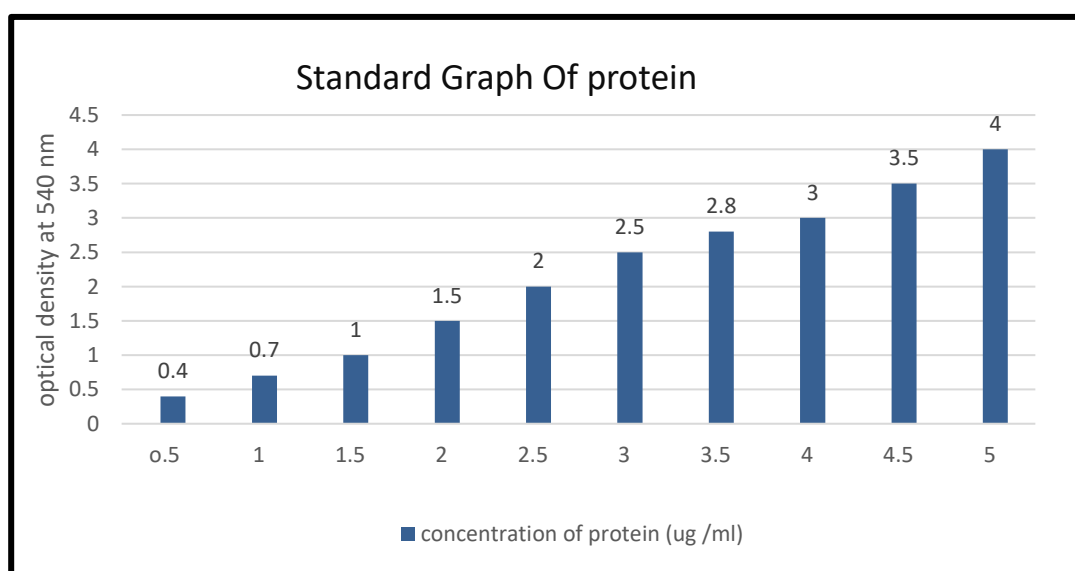
❖ Fish Scale Degradation Study

➤ Estimation of Protein:

Protein Estimation was carried out by using the biuret method. The optical density was measured at 540 nm. The results showed that optical density increased with increase in protein concentration, indicating a direct proportional relationship. (Table No :14)

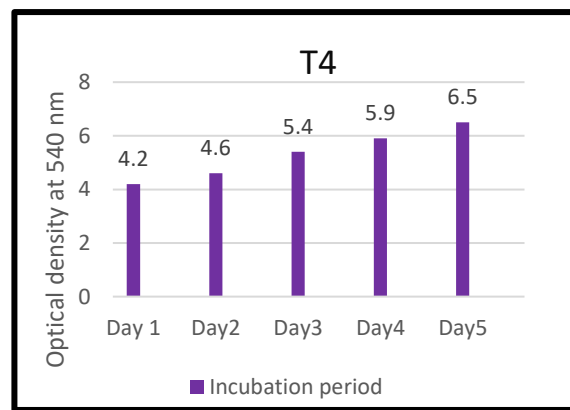
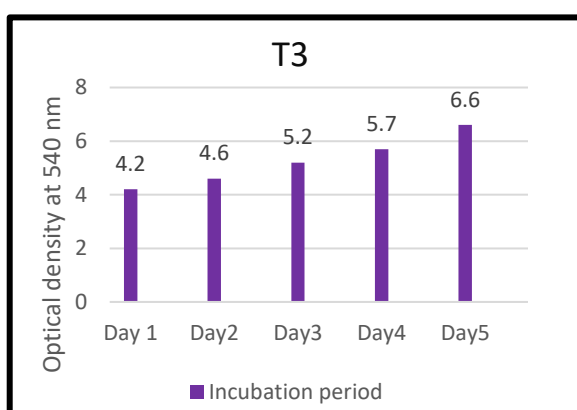
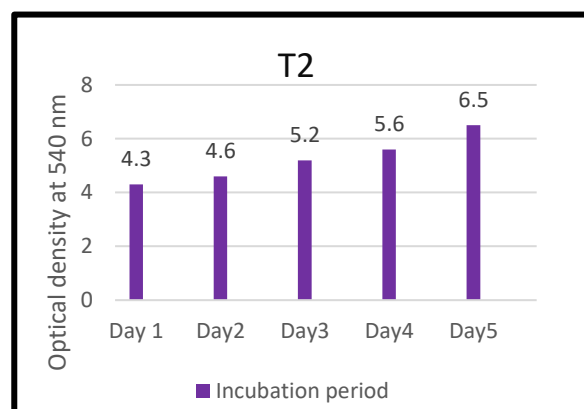
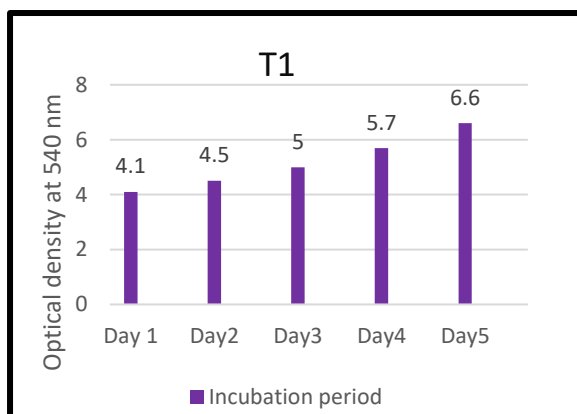
Table No :14 Standard protein concentration

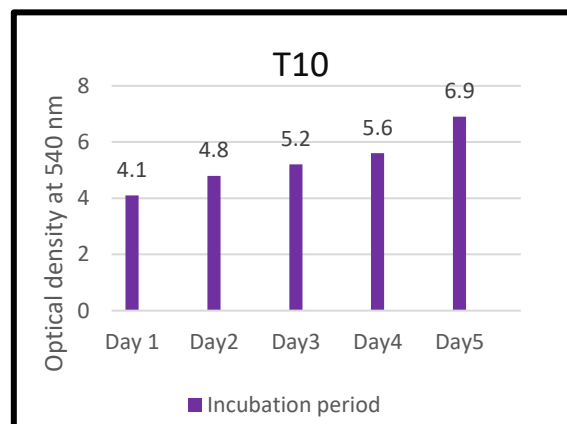
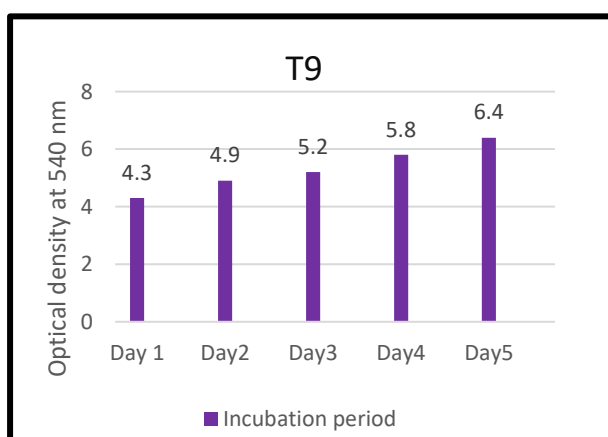
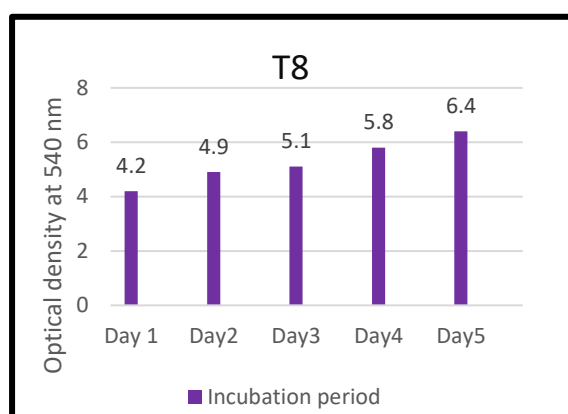
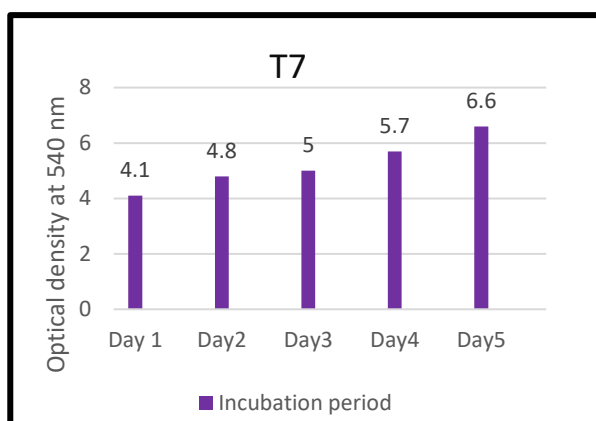
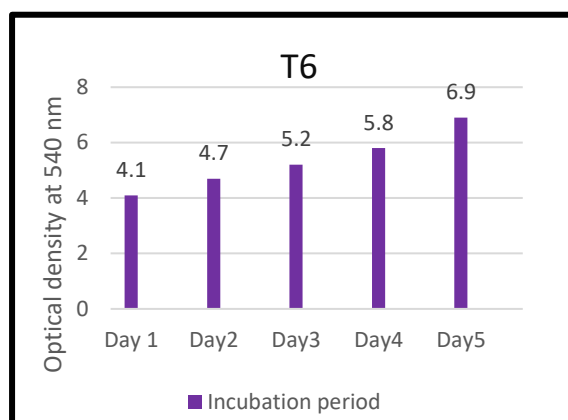
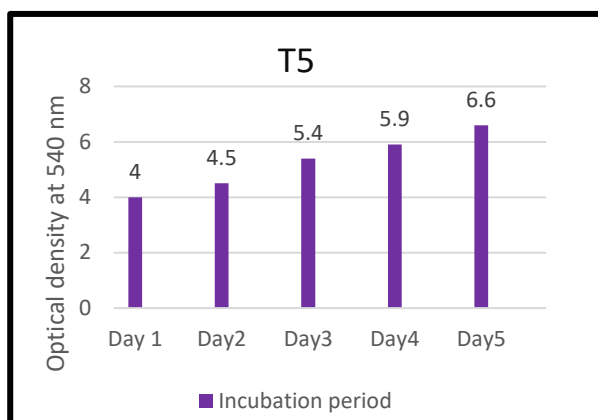
| Sr. No | Concentration of Protein (ug/ml) | Optical Density at 540nm |
|--------|----------------------------------|--------------------------|
| 1 | 0.5 | 0.1 |
| 2 | 1.0 | 0.7 |
| 3 | 1.5 | 1.0 |
| 4 | 2.0 | 1.5 |
| 5 | 2.5 | 2.0 |
| 6 | 3.0 | 2.5 |
| 7 | 3.5 | 2.8 |
| 8 | 4.0 | 3.0 |
| 9 | 4.5 | 3.5 |
| 10 | 5.0 | 4.0 |



| Bacterial isolates | Optical Density at 540 nm | | | | |
|--------------------|---------------------------|--|--|--|--|
|--------------------|---------------------------|--|--|--|--|

| | Day1 | Day2 | Day3 | Day4 | Day5 |
|-----|------|------|------|------|------|
| T1 | 4.1 | 4.5 | 5.0 | 5.7 | 6.6 |
| T2 | 4.3 | 4.6 | 5.2 | 5.6 | 6.5 |
| T3 | 4.2 | 4.6 | 5.2 | 5.7 | 6.9 |
| T4 | 4.1 | 4.8 | 5.4 | 5.9 | 6.5 |
| T5 | 4.0 | 4.5 | 5.4 | 5.7 | 6.8 |
| T6 | 4.1 | 4.7 | 5.2 | 5.8 | 6.6 |
| T7 | 4.1 | 4.8 | 5.0 | 5.7 | 6.8 |
| T8 | 4.2 | 4.9 | 5.1 | 5.9 | 6.7 |
| T9 | 4.3 | 4.9 | 5.2 | 5.8 | 6.4 |
| T10 | 4.1 | 4.8 | 5.2 | 5.6 | 6.7 |

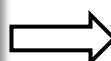
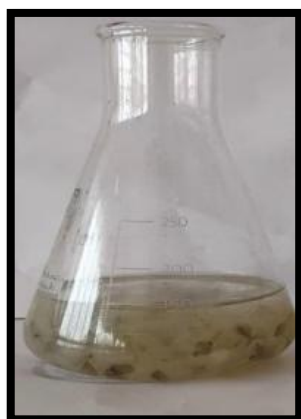




The Protein concentration of unknown samples (T1-T10) was estimated using optical density measurement at 540 nm over a period of five days. The results showed a gradual increase in optical density values from day 1 to day 5 for all samples. Among all isolates, T6 and T10 show comparatively higher OD values. Overall, the results indicate that all bacterial isolates demonstrated the ability to utilize protein over time.

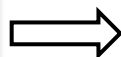
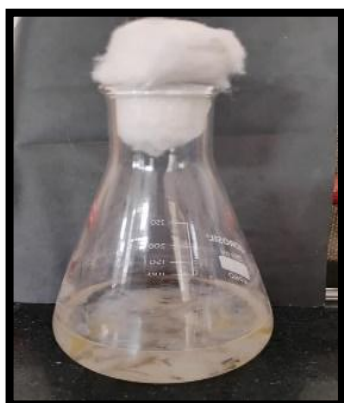
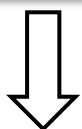
❖ Gelatin Extraction From Fish Scale

Fish Scale was processed by defatting, demineralization and acid treatment followed by extraction at 50- 70°C & Gel Formed After 24 hours at 4°C. Thus, Gelatin was extracted from fish scales with good gel formation. The clarity and consistency of gel suggested good quality gelatin.



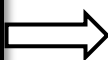
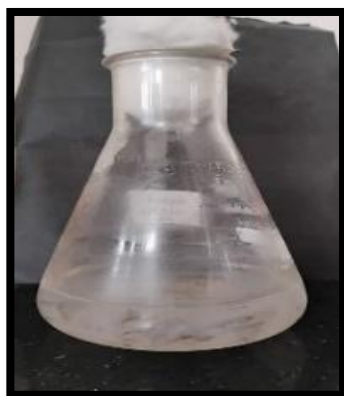
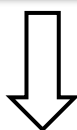
1) Collection Of Fish Scale –

Fish Scales were Collected and cleaned properly.



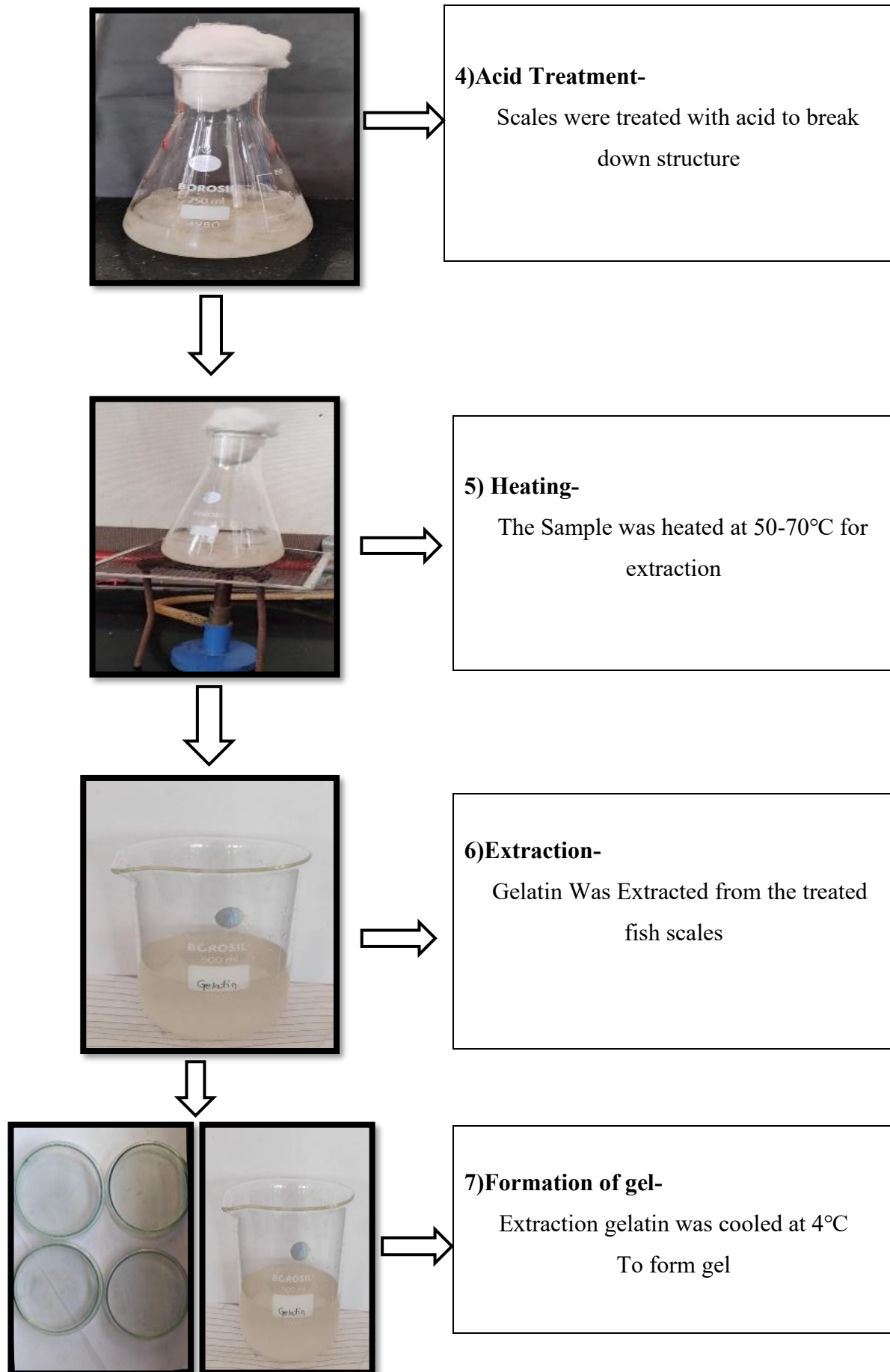
2) Defatting-

Scales were treated to remove fats and impurities.



3) Demineralization-

Minerals were removed using Suitable Chemicals.

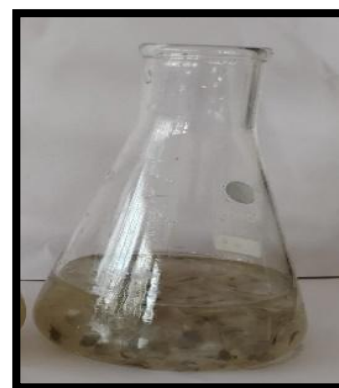
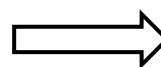


Preparation of Bioplastic Film

Bioplastic film was successfully prepared from fish scale gelatin using alkali treatment and heating. The obtained film appeared flexible, and semi-transparent, indicating good film-forming ability. This confirms that fish scale gelatin can be used as a biodegradable and eco-friendly alternative to conventional plastics.

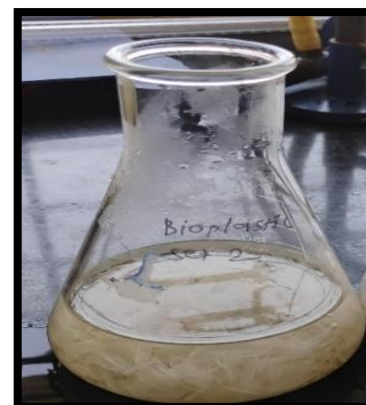
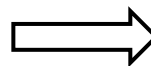
1) Fish scale collection

Fish Scales were collected, thoroughly washed and cleaned to remove dirt and impurities



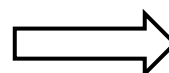
2) Alkali Treatment

The cleaned fish scale was treated with an alkali solution to remove non-collagenous protein and unwanted material



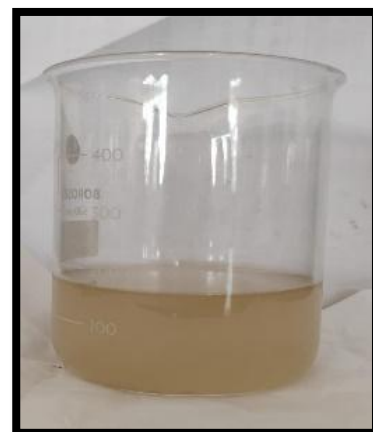
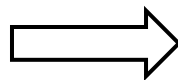
3) Heating

Scales were heated at 60-70°C for 3-4 hrs



4) Extraction

The solution was filtered to remove impurities and glycerol was added as a plasticizer and mixed well

**5) Bioplastic film**

The dried biodegradable bioplastic film was obtained



❖ Tests for Physical Evaluation of bioplastic

1) Water Absorption Test (Hydrophilicity Test)

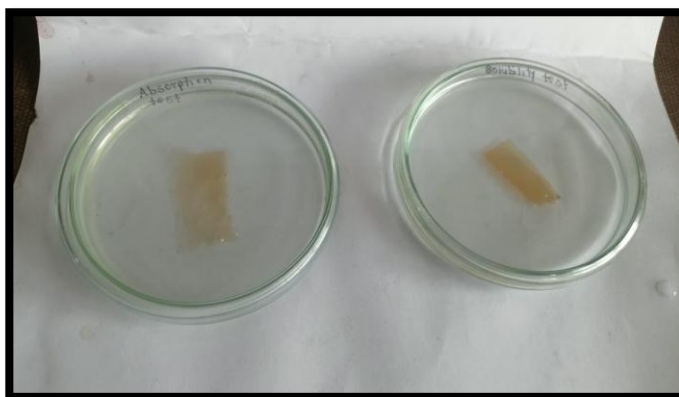


Fig No12: Water Absorption Test

The Bioplastic sample showed extremely high-water absorption and completely disintegrated after 24 hours of immersion. This indicates that the material is highly hydrophilic and water – Soluble in nature.

2) Solubility Test

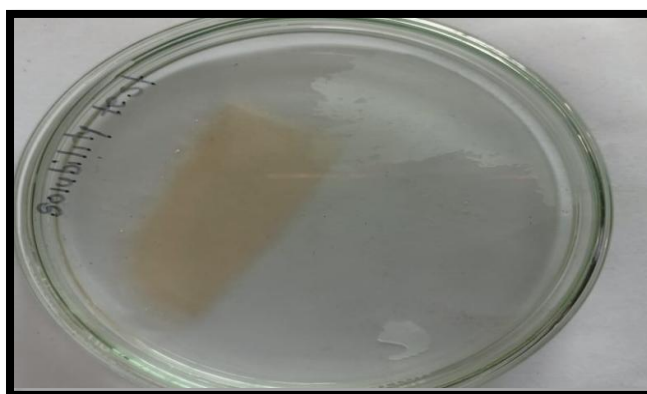


Fig No13: Solubility Test

The Bioplastic sample completely dissolved in water within 24 hours, indicating high solubility. This suggests that the material is suitable for applications where rapid degradation is required, but not for long term water exposure.

3) pH stability Test (Acid & Alkali)



Fig No14: pH stability Test

The Bioplastic sample showed complete degradation in both acidic and alkaline conditions indicating low chemical stability. This suggests that the polymer structure is sensitive to pH changes and undergoes rapid breakdown.

4) Burning Test

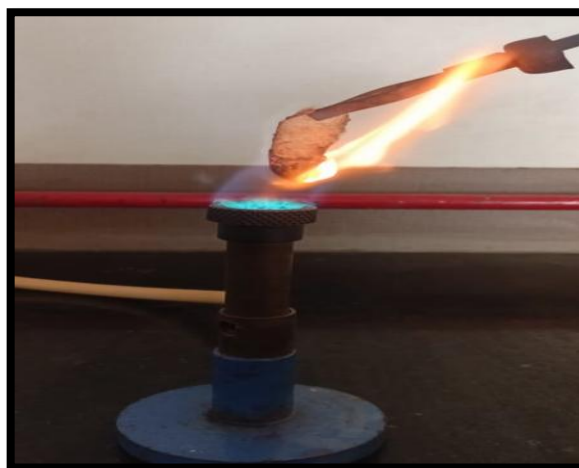


Fig No15: Burning Test

The Bioplastic sample burned with characteristics similar to paper and produced fine, powdery ash. This indicates the presence of natural polymer components such as starch or cellulose confirming its biodegradable nature

5) Heat resistance Test result

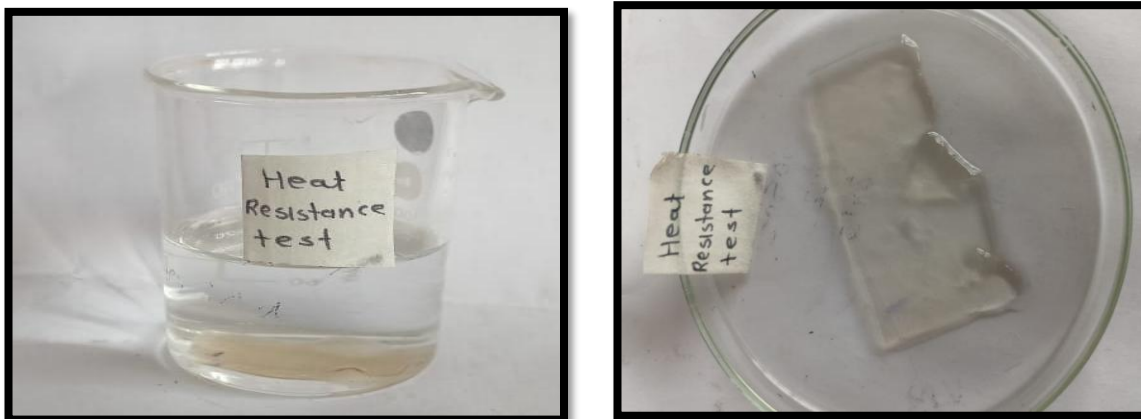


Fig No16: Heat Resistance Test.

The bioplastic remained intact without melting at 60°C for 10 minutes, showing good heat resistance, with slight cloudiness indicating minor structural change.

6) Transparency Test

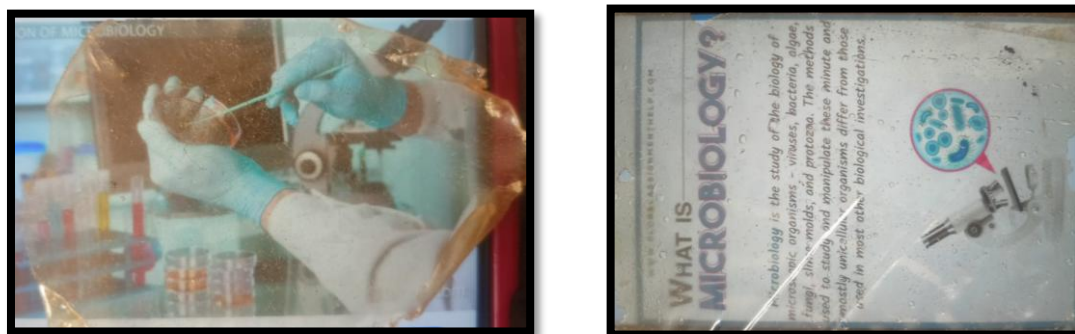


Fig No17: Transparency Test

The Bioplastic sample showed partial transparency, allowing light to pass through but not clearly, this indicates a semi-transparent nature, similar to low-density plastic materials. Hence, the bioplastic can be suitable for applications where moderate transparency is acceptable.



DISCUSSION

Discussion

Nowadays bigger problems are environmental concerns with increasing pollution day by day, so while looking for it we found the Fish processing generation in a considerable amount of solid waste of fisheries such as skin, bones, fins, and scales. Among these by-products of fish, fish scales represent a significant portion and are often discarded without proper utilization in local areas near fish market or industries leading to environmental concerns. In recent years, the valorization of fish processing waste has gained attention as a sustainable strategy for converting waste into valuable biomaterials. Gelatin obtained from fish sources has become an important alternative to mammalian gelatin due to its biodegradability, lower risk of disease transmission, and wider cultural acceptance (Karim & Bhat, 2017).

However, several studies have reported that fish scales are a rich source of collagen, which can be effectively converted into gelatin through controlled chemical and thermal treatments. For instance, Karim and Bhat (2017) highlighted the potential of fish-derived collagen as an alternative source for gelatin production, while Liu et al. (2019) demonstrated efficient extraction of collagen from fish scales for biomaterial applications. These findings helped us for doing research on - use of fish scales as a suitable raw material for gelatin production.

In addition to gelatin extraction, the present study also focused on the isolation of protease-producing microorganisms from soil samples collected near fish market areas of Pethvadagon, kuditre, N.P.fish market dumping area kolhapur, shiroli, shigaon ,koge dam .In the present study, we focus on gelatin extraction from fish scales using alkaline and acid pre-treatment followed by hot water extraction at 60–70°C. The extracted gelatine exhibited a slightly yellow to whitish colour and transparent gel-like texture, indicating successful conversion of collagen to gelatin. Similar observations were reported by (Nurilmala et al., 2018), who found that gelatin extracted from fish scales initially appears slightly yellow due to residual pigments but becomes lighter after purification. Furthermore (Liu et al.,2019) reported that fish scale gelatin typically forms a semi-transparent gel structure when cooled, which is consistent with the results observed in the present study.

The solubility and texture of the gelatin obtained in this study also confirm its typical physicochemical properties like solubility test- as while doing this test

we found that gelatin is dissolved in warm water and form a gel upon cooling which confirms partial renaturation of collagen derieved polypeptide chains. Previous studies have demonstrated that fish-derived gelatin possesses good gel-forming ability and viscosity, making it suitable for applications in food processing and pharmaceutical formulations (Ahmad & Benjakul., 2019). Also the absence of strong fishy odor in the extracted gelatin further indicates that washing and pre-treatment steps effectively removed impurities and non-collagenous proteins from the fish scales.

The serial dilution and plating on sterile nutrient agar medium were used for isolating bacteria capable of degrading protein substrates such as fish scales. Environmental samples including soil, water, and fish scales are known to harbor diverse microbial populations capable of producing extracellular enzymes involved in organic matter degradation. (Sharma et al., 2017). Microorganisms were isolated using the serial dilution technique followed by plating on nutrient agar medium, which supports the growth of heterotrophic bacteria. A total of 20 bacterial isolates were obtained (as A1 to A5, B1-B10, C1-C6,) and characterized based on colony morphology. Most colonies appeared circular with smooth, entire margins, and the colonies were predominantly white, yellow or opaque in appearance. These morphological characteristics are commonly observed among environmental bacterial isolates and indicate the presence of diverse microbial communities capable of surviving in nutrient-rich environments. (Khan & Malik, 2018). The isolation of microorganisms from fish scale-associated environments suggests that these habitats may contain bacteria capable of producing proteolytic enzymes required for the degradation of protein substrates such as collagen and casein (Sharma et al., 2017).

The isolated bacteria were further characterized using Gram staining and motility tests. Gram staining results indicated that most of the isolates were Gram-positive, and microscopic observations revealed the presence of cocci and short rod-shaped bacterial cells. Gram-positive bacteria are widely reported to produce extracellular enzymes such as proteases that play a crucial role in the degradation of complex protein substrates. (S Madigan et al., 2018) Motility tests were also conducted to determine the movement ability of the isolates.

Furthermore the biochemical characterization of the 11 selected isolates was performed using the IMViC test series, including Indole, Methyl Red (MR), Voges–Proskauer (VP), and Citrate utilization tests. The results indicated that most of the isolates were indole negative (T1, T2, T3, T4, T5, T6, T7, T9, T10), while 2 isolates (T3 & T10) showed positive reactions. Some isolates also showed positive MR (T3, T8, T9) and VP (T2, T8, T9) results, indicating variations in fermentation metabolism among the bacteria. These biochemical differences suggest the presence of metabolically diverse bacterial isolates, which may contribute to their ability to degrade protein-rich substrates such as fish scales. (Cappuccino & Sherman et al, 2017)

In the present study, we focus on gelatin extraction from fish scales using alkaline and acid pre-treatment followed by hot water extraction at 60–70°C. The extracted gelatine exhibited a slightly yellow to whitish colour and transparent gel-like texture, indicating successful conversion of collagen to gelatin. Similar observations were reported by (Nurilmala et al., 2018), who found that gelatin extracted from fish scales initially appears slightly yellow due to residual pigments but becomes lighter after purification. Furthermore (Liu et al., 2019) reported that fish scale gelatin typically forms a semi-transparent gel structure when cooled, which is consistent with the results observed in the present study.

The solubility and texture of the gelatin obtained in this study also confirm its typical physicochemical properties like solubility test- as while doing this test we found that gelatin is dissolved in warm water and form a gel upon cooling which confirms partial renaturation of collagen derived polypeptide chains. Previous studies have demonstrated that fish-derived gelatin possesses good gel-forming ability and viscosity, making it suitable for applications in food processing and pharmaceutical formulations (Ahmad & Benjakul, 2019). Also the absence of strong fishy odor in the extracted gelatin further indicates that washing and pre-treatment steps effectively removed impurities and non-collagenous proteins from the fish scales.

Following gelatin extraction, the fish scales were further utilized for the preparation of biodegradable bioplastic films. In the present study, the fish scales in specific quantity was treated with 0.1 N Naoh solution for 24 hours period of time and subjected with distilled water for heat treatment with use of water bath and mixed with a small quantity of glycerol, which acted as a plasticizer during film formation. Plasticizers such as glycerol are commonly incorporated into gelatin-based films to improve flexibility, reduce brittleness, and enhance mechanical stability of the polymer matrix. After mixing with glycerol, resulting into the formation of a 2mm thick transparent bioplastic film. The transparency of this film indicates uniform distribution of polymer chains. (Zhang et al., 2019) Also the film were showed 2 different colours as oberseved for fish scales from different local areas as in white and transparent brownish colours. Gelatin-based bioplastics are considered promising alternatives to petroleum-based plastics because they are biodegradable, renewable, and environmentally sustainable. Such biodegradable films have potential applications in food packaging, biomedical materials, and eco-friendly polymer industries (Tharanathan et el.,2018).

In the present study, 10 isolates were screened for protein degradation, among which three isolates showed promising degradation activity within one week of incubation at room temperature. Although the zone diameter of hydrolysis was not measured in this study we only focus on presence of degradation ability, the protein degradation ability was further confirmed through biochemical analysis. Protein degradation activity was further analyzed using the Biuret method (1gm fish scale + Minimal media + culture isolates) which is a colorimetric assay used to detect peptide bonds in proteins under alkaline conditions. the intensity of colour was measured spectrophotometrically resulting values in increasing manner. In the present study, optical density values were recorded at 540 nm, and the observed absorbance values ranged from 0.10 to 0.29, indicating the presence of soluble protein fragments generated during microbial degradation. These variations in optical density reflect differences in protein concentration resulting from enzymatic activity of the bacterial isolates. (Khan & Malik, 2018).



CONCLUSION

Conclusion

The present study successfully demonstrated the potential of fish scale waste as a valuable resource for sustainable biomaterial production and environmental management. Fish processing industries generate a significant amount of solid waste, particularly fish scales, which are often discarded without proper utilization, leading to environmental concerns. However, this study highlights that such waste materials can be effectively converted into useful products, thereby contributing to waste valorization and circular bioeconomy approaches.

Gelatin extraction from freshwater fish scales was carried out using alkaline and acid pretreatment followed by hot water extraction at 60–70°C, which proved to be an efficient and relatively simple method. The extracted gelatin exhibited desirable characteristics such as slightly yellow to whitish colour, transparency, and gel-forming ability upon cooling, confirming the successful conversion of collagen into gelatin. The observed solubility in warm water and gel formation upon cooling further validated its typical physicochemical properties. Additionally, the absence of strong fishy odor indicated effective removal of impurities during pretreatment, suggesting that the adopted method is suitable for obtaining good-quality gelatin with minimal processing complexity.

Furthermore, the extracted gelatin was successfully utilized for the preparation of biodegradable bioplastic films, demonstrating its potential application as an eco-friendly alternative to conventional plastics. The addition of glycerol as a plasticizer improved the flexibility and structural integrity of the films. The formation of transparent films with uniform texture indicated proper polymer interaction and distribution. The variation in film colour observed from different fish scale sources suggests that raw material composition may influence the final properties of the bioplastic. Overall, the results confirm that fish scale-derived gelatin can serve as a promising material for the development of biodegradable and sustainable packaging solutions.

In addition to biomaterial development, the study also explored the microbial aspect of fish waste degradation. A total of 20 bacterial isolates were

obtained from soil, water, and fish scale samples, indicating rich microbial diversity in such environments. Morphological and biochemical characterization revealed that most isolates were Gram-positive and exhibited diverse metabolic characteristics, as confirmed by IMViC test results. This diversity suggests the presence of bacteria with varied enzymatic capabilities.

Among them 10 isolates were screened for protein degradation, and three isolates showed significant degradation activity within one week. This indicates the presence of protease-producing microorganisms capable of breaking down protein-rich substrates such as collagen present in fish scales. The degradation ability was further confirmed using the Biuret method. These findings highlight the potential of naturally occurring microorganisms in the biodegradation and recycling of organic waste materials.

Overall, this study integrates gelatin extraction, bioplastic production, and microbial protein degradation, providing a comprehensive approach to fish waste utilization. The results emphasize that fish scale waste can be transformed into value-added products such as gelatin and biodegradable films while also supporting microbial processes that contribute to waste degradation. Such integrated strategies can play a significant role in reducing environmental pollution, promoting sustainable resource utilization, and advancing eco-friendly material development.



BIBLIOGRAPHY



Referances :

1. Ahmad, T., et al. (2024). Microbial extraction of gelatin from fish waste. *Journal of Biotechnology*.
2. Arvanitoyannis, I. S., & Kassaveti, A. (2008). Fish waste utilization. *Waste Management*.
3. Benjakul, S., & Morrissey, M. T. (2005). Fish gelatin properties. *Food Hydrocolloids*.
4. Bourne, M. C. (2002). Food texture analysis. *Food Technology*.
5. Cole, C. G. B. (2000). Gelatin functionality. *Food Science Journal*.
6. FAO. (2022). State of world fisheries.
7. Ghaly, A. E., et al. (2013). Fish waste management. *American Journal of Environmental Science*.
8. Glicksman, M., et al. (1969). Hydrocolloids in food. *Food Technology*.
9. Gomez-Guillen, M. C., et al. (2007). Fish gelatin applications. *Food Hydrocolloids*.
10. Gomez-Guillen, M. C., et al. (2011). Gelatin functional properties. *Food Hydrocolloids*.
11. Jafari, H., et al. (2020). Fish scale collagen. *Marine Drugs*.
12. Karim, A. A., & Bhat, R. (2009). Fish gelatin review. *Food Hydrocolloids*.
13. Kim, S. K., & Mendis, E. (2006). Marine by-products. *Food Research International*.
14. Lobo, A., et al. (2002). Emulsifying properties of gelatin. *Food Chemistry*.
15. Lu, J., et al. (2018). Nutritional value of gelatin. *Journal of Nutrition*.
16. Luo, Q., et al. (2020). Fish scale composition. *Marine Science Journal*.
17. Meena, C., & Deshpande, S. (1999). Collagen structure. *Biochemical Studies*.
18. Mirzapour Kouhdasht, A., et al. (2018). Enzymatic gelatin extraction. *Food Chemistry*.
19. Mohanty, B. P., et al. (2019). Nutritional composition of fish. *Food Chemistry*.
20. Nagai, T., & Suzuki, N. (2000). Collagen extraction. *Food Chemistry*.
21. Norziah, M. H., et al. (2009). Gelatin extraction methods. *Food Chemistry*.

22. OECD-FAO. (2023). Agricultural outlook.
23. Rustad, T. (2003). Fish gelatin characteristics. *Food Research International*.
24. Shahidi, F., & Ambigaipalan, P. (2018). Bioactive compounds. *Journal of Functional Foods*.
25. Shahidi, F., & Synowiecki, J. (1991). Fish gelatin. *Journal of Food Science*.