

BIOREMEDIATION OF SEAFOOD PROCESSING PLANT EFFLUENT: COLLECTION AND BIOCHEMICAL ANALYSIS OF EFFLUENT

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Abstract

Microorganism bioremediation of seafood processing plant wastewater is a viable alternative to the traditional effluent treatment technique of recovery and removal. Five seafood processing plants in and around Veraval were sampled for waste water. This water was transported to lab in controlled condition and were analysed for eutrophic component present and BOD, COD value evaluation. Soil samples from the same places were collected for bacterial isolation. From them over 40 bacterial strains were collected from five different seafood processing industry soil samples near Veraval.

Keywords: *bioremediation, effluent treatment, eutrophic componenet degradation, bacterial isolates, IMViC test*



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Introduction: The world population is expected to continue to grow, which means that the demand for food, water, and energy is expected to climb significantly. The availability of fresh water is critical to the sustainability and safety of food. The utilization of fresh water in current food production systems is a major issue, as is the environmental impact of the sector. Global fresh water withdrawals, which include agriculture and livestock production, are estimated to account for approximately 60% of the world's water use. In the next 30 years, freshwater consumption for food and livestock production is expected to rise by 65 percent. Wastewater from the processing of food contains a wide range of nutrients and other important components. Wastewater discharged carelessly and without treatment is a leading cause of pollution and other harmful effects on the environment. More than half of the biodegradable organic matter in one-third of the food produced worldwide (1.3 billion tons) is lost or abandoned, according to a recent estimate [2]. Proper treatment of wastes, including effluents, is one way to combat pollution while simultaneously preventing the loss of food

constituents during manufacture of clean food. Increasing food production's overall economic and environmental sustainability by recovering resources from waste streams is possible.

In order to offer consumers with a wide variety of fish and shellfish, the seafood business relies on both traditional fishing methods and more modern farming techniques. 178.5 million tons of seafood were produced around the world in 2018. (MT). Fish like tuna, herring, and mackerel, crustaceans like shrimp, krill, crab, and lobster, bivalves like mussels and oysters, and cephalopods like squid and cuttlefish are all examples of marine seafood. At 82.1 MT in 2018, aquaculture production has expanded at a pace of 5.8 percent per year since 2001. Carp, tilapia, and shellfish are common farmed in fresh water, whereas Atlantic salmon, sea bsssss, and sea bream are farmed in salt water. Products such as chilled, frozen, smoked, dried, fermented or marinated or other commodities make up roughly 78% of the industry's entire crop. Chilled fish, finfish steaks, shrimp goods, and squirmy (mechanically deboned, water washed minced fish meat) are some of the most popular consumer products that are traded in a wide variety of marketplaces around the world. Both aquaculture and capture fisheries play complimentary roles in enhancing the availability of seafood for nutritional and health gains.

In spite of this, the available seafood isn't all being utilized. By-catch, garbage, and waste on land all contribute to a huge number of wastes. About 40% of raw material is used to make products such skins, heads and frames; viscera; fillet cut-offs; and others. Offal production is being bolstered by the expansion of aquaculture plants. An estimated 8% of the world's seafood production is lost each year, totaling 7.3 metric tons (MT) between 1992 and 2001. Putrefaction is much quicker in fish waste than in municipal garbage because of the high protein and other nitrogenous content. On a dry weight basis, the average protein content in seafood wastes is 60 percent, the fat content is 19 percent, and the ash content is 21 percent. Increasing fishing wastes, particularly effluents, pose expanding environmental challenges as a result of the growing seafood industry. Solid waste is often disposed of in one of three ways: in a landfill, as silage, fishmeal, or fertilizer, or as a component of animal or poultry feed, depending on the material.

Aquaculture water use:

The fishing sector relies heavily on water. Capture fishing, cage and pen aquaculture, and the harvesting of fry or juveniles for aquaculture all make use of fresh, brackish, or saline surface waters to offer fish habitats. For aquaculture to be successful, a substantial amount of fresh water is needed. The amount required depends on the type of aquaculture system used, the

species being farmed, and the location of the farm. Due to evaporation, farm water is also lost. Sea bass farmed in cages can use up to 48, 782 m³ of water per ton, while spiny lobsters use only 1.5 m³ and generic fish only 6 m³. Indirect uses of water include farming, aqua feed production, and sanitation and hygienic maintenance of machinery and equipment, to name a few. Cleaning, beheading, filleting, scaling, peeling, frying, icing, and other pre-processing activities necessitate the use of water, as is the use of cold chain systems to keep fishery products fresh throughout the handling process. Storage and transportation of raw and finished products also necessitates the use of water. Approximately 10–40 m³ of fresh water per tonne of processed raw material is used in the processing process. According to India's current industrial water pricing, this comes to about \$22 USD. Because surimi production involves a lot of washing, it uses more water than other processes like canning and curing. Marinated herring or peeled shrimp take about 7,000–8,000 and 50,000 liters of water, respectively, to create one ton of each. There is a constant flow of effluents from the boiling, filleting, and marination processes. Over 1500m³ of wastewater is produced daily by one of Europe's largest herring processing plants, which processes about 50,000 tons of fish each year. Figure 1 depicts the effluent generation process used in the seafood processing industry. There is a worldwide worry about the amount of water used in the fish processing sector and the amount of highly concentrated post-processing wastewater that is discharged. Despite the fact that the seafood sector, including aquaculture, uses a lot of water, the cost-benefit analysis of that water has gotten little attention. As the global demand for seafood rises and aquaculture becomes one of the most rapidly expanding animal food industries, the 'seafood gap' in the food-water nexus is expected to become increasingly problematic. This necessitates water conservation measures.

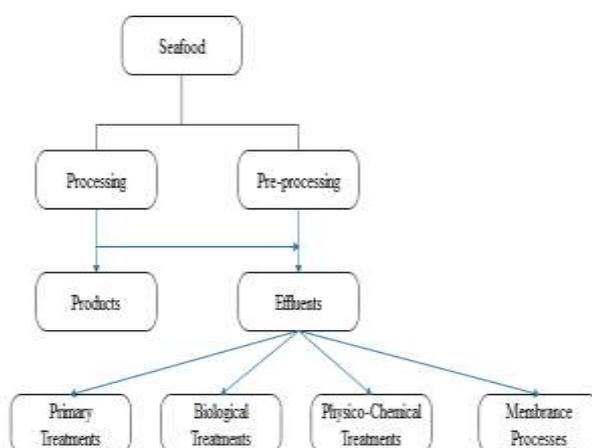


Fig1: Effluent generation during seafood processing

Waste water from the manufacture of fish meal is known to have a high organic load because of this. Surimi washing wastewater has also been found to have a pretty significant organic load. Bioremediation is not always possible due to the presence of diverse bacteria in wastewater. There must be a high-quality, fast-growing inoculum that can suppress the growth of heterotrophic microorganisms in wastewater to prevent contamination. Fish and marine invertebrates have a lot of protein, which raises nitrogen levels in the water. In processing plants, raw materials have a significant impact on effluent properties. When it comes to the quality of raw materials, time has also been shown to have an effect on their quality while nitrates and phosphates are commonly found in fish waste, their concentrations are usually quite low. Phosphorus can also be introduced into the food chain through the use of processing and cleaning chemicals. Fish meal production wastewaters are known to be diluted with cooling water from the overall process prior to disposal if they contain high concentrations of toxins.

Treatment of seafood industry effluents:

Regulatory bodies have imposed increasingly strict standards on effluent treatment, particularly in terms of BOD, COD, and TSS values. Effective treatment is mostly determined by the effluent type and quality (BOD/COD/TSS/FOG and the BOD/COD ratio). The influent volume also plays a significant role in determining the most appropriate treatment. Sustainable seafood processing, clean air, and water conservation are all tied to the issues of wastewater treatment.

Primary treatments, such as screening and sedimentation, are first applied to the effluents. Larger particles, such as bones and shells, are removed by screening. The nitrogen load was lowered by pretreatment with a 0.8 mm filter and a two-stage flotation process. lipids and hydrophobic sludge can form in the presence of FOG, causing blockage and hindering sedimentation. It was possible to remove up to 40% of the suspended particles by using a decanter as a physical pre-treatment device. The sludge removal system guarantees that settled sludge is quickly removed while causing the sludge blanket as little disruption as possible. Physio-chemical, biological, membrane, and other methods can be used to treat fish waste.

- **Physicochemical methods:** There are a variety of physicochemical treatment options, such as pH adjustment, coagulation, flocculation, chemical precipitation, chemical oxidation (such as ozonation, UV irradiation, halogenation), electrolysis, polymeric adsorption, granular activated carbon adsorption, dissolved air flotation (DAF), sludge

treatments, and more.. When using bactericidal chemicals like chlorine or ozone (O₃) or ultraviolet (UV) radiation, effluents are disinfected. Hydrogen peroxide (H₂O₂) treatment with ozonation or UV irradiation leads to more oxygen-rich, less hydrophobic, and more biodegradable organic material in effluent streams. FOG and BOD can be effectively reduced through the use of coagulation, flocculation, flotation, and emulsification techniques. Coagulation and flocculation can go more smoothly if colloidal particles are lowered in pH, which removes charges from them. Anionic and cationic polyelectrolytes can be added to the protein suspension to aid in protein aggregation.

- **Biological treatment:** Microorganisms such as bacteria, fungus, and protozoa are used to breakdown organic materials in the effluents using aerobic or anaerobic conditions. Activated sludge systems, aerobic lagoons, trickling filters, and rotating disc contactors are the most prevalent aerobic processes. It is typical practice in activated sludge treatment systems to use an optimal, mixed flora of microbes to decompose organic waste materials in the presence of excess dissolved oxygen and nutrients, including nitrogenous chemicals. To reduce soluble BOD₅, lipids, and nutrients, the treatment is particularly effective in this regard.
- **Membrane processes:** Organic matter separation using membrane-based separation processes (MBSPs) is a relatively new development. For biowaste, MBSPs can also remove pathogens like bacteria and viruses. It is important to note that the key MBSPs are ultrafiltration and nanofiltration (UF), as well as microfiltration and MF (FO). MF, UF, NF, RO, and FO approaches, as well as hybrid technologies, offer a good chance of providing clean water from wastewater streams at a low cost and with minimal energy use. Water softening, wastewater treatment, vegetable oil, and other industries could benefit from the use of NF. Wastewater treatment systems, both municipal and industrial, are now using hybrid RO/NF membranes.
- **Combination processes:** Most effluents can't be successfully treated with a single technique in most cases. A synergistic effect can be achieved by combining several processes to improve efficiency, quality, yield, and costs. The COD in the effluents of tuna processing plants was reduced by up to 95% using an integrated system that included a decanter to remove lipids and TSS, an anaerobic digester, and an activated sludge aerated bio-reactor, whereas individual processes were only moderately successful in reducing the COD.

- **Treatment of aquaculture effluents:** Aquaculture requires careful attention to avoid environmental dangers, such as the loss of fish species and a possible decrease in aquatic productivity, in the farm waters. This is necessary for sustainable aquaculture. In order to improve the effluent quality, it is necessary to follow best practices in pond building, feed selection, proper feeding of fish and shellfish, erosion management, moderate stocking densities, and the use of settling basins. These include filtration and mechanical separation as well as sedimentation, filtration, chemical and biological treatments.

Resource recovery from effluents:

Food, pharmaceutical, and other businesses could benefit from the valuable components found in seafood processing wastes and waste streams. In addition to protecting the environment, the utilization of fishing wastes can have a significant impact on marine resource conservation and product development costs. Seafood solid discards and wastewater must be acknowledged as sources of diverse components, water, and energy in order for seafood processing to be sustainable. Bio-compounds that can be recovered from food process effluents can be useful in nutrition, healthcare, pharmaceutical, and other industries, as well as in reducing environmental dangers. There is a major advantage to using fish byproducts for valorisation, despite the fact that both processes have the same environmental impact. As a contrast to effluents, the bioactive compounds found in solid wastes such as fish oils, proteins and peptides; collagen; gelatin; enzymes; chitin; and minerals have received more attention as sources of high-value bioactive ingredients. Because of advancements in green technology and biotechnology, it has become more easier to isolate these chemicals. Microwave, ultrasound, super-critical fluid extraction, enzyme extraction, fermentation-dependent extraction, membrane-filtration, and other techniques are among the possible methods of extraction. The U.S. Environmental Protection Agency (EPA), which focuses on recycling resources in addition to treating waste goods, remarked that the high quantities of nitrogen and phosphorus in wastewater might be used as nutrient resources for agriculture. Recovering nutrients, reusing water, and harnessing energy from effluents through holistic secondary processing might help maximize the benefits of marine resources.

A. Food ingredients:

Seafood business effluents contain a wide range of important substances for the food industry. Fishery effluents should be used as soon as possible, since the longer they are held, the more BOD and COD they contain, which reduces the amount of high-quality products that may be recovered.

B. Argo-industry applications

Proteins can be recovered from effluent treatment sludge by disintegration, alkali treatment, ultrasonication, precipitation, and drying. Aflatoxin B1 and ochratoxin A were eliminated by this method. The proteins could be employed as a safe and nutritious animal feed that is comparable to commercial protein diets in terms of nutritional value. When applied to the soil and the plants, bio-stimulants aren't fertilizers, but rather aid in nutrient uptake by enhancing metabolic processes.

C. Bio-energy

Interest in biological materials as new sources of energy has grown as a result of the global concern over energy security. There is a lot of emphasis on biogas, bio-hydrogen and bioethanol as well as the production of biodiesel in the bioenergy sector. Methanogenic bacteria may be inhibited by the sludge's high concentrations of light metals and fats and proteins.

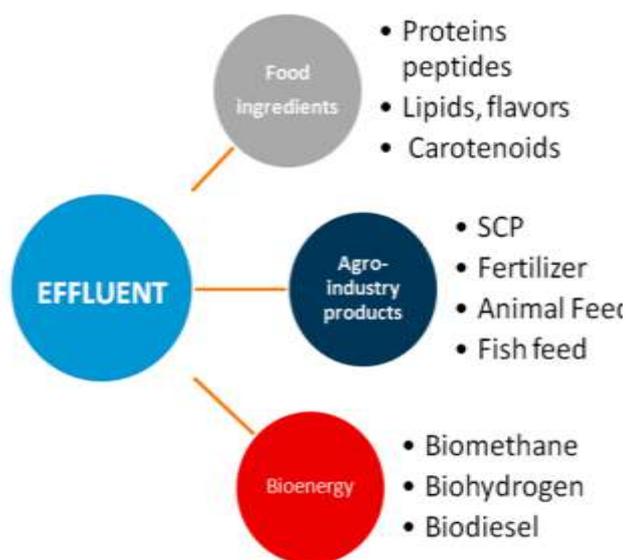


Fig2: Products recovered from seafood effluents.

MATERIALS AND METHODS

A. Collection, Isolation and Identification of bacterial strains:

It was necessary to collect effluent samples from five different seafood processing plants in and around Veraval and bring them to the lab in an ice box for testing. Physical and chemical properties of the sample were analyzed in the beginning. Samples from this collection were utilized to isolate and identify microorganisms that could be employed as bioremediators in the future. Incubation was place at 30oC for 48 hours with serially-diluted samples plated onto nutrient agar plates. The visual, biochemical, and molecular characteristics of isolated

bacterial colonies cultured on agar plates were used to sort them. A variety of biochemical tests were used to help pinpoint the isolates. This included Gram stains, Catalase testing, Indole production testing, Starch hydrolysis testing, Triple Sugar Iron agar (TSI) testing, Methyl Red testing, Voges-Proskauer testing, Nitrate testing, Citrate testing, the Oxidative Fermentation testing, and the motility testing. It was possible to identify the genomic strain using 16S rDNA.

RESULTS AND DISCUSSION:

Ammonia (NH₃-N), nitrite (NO₂-N), nitrate(NO₃-N), phosphate(PO₄-P), chemical oxygen demand, and biochemical oxygen demand were all used to evaluate the effluent quality of a seafood processing plant. The average ammonia (NH₃-N) concentration was 9.12 mg/l in the wastewater from the seafood processing plants. The average concentration of nitrite (NO₂-N) was 93.48 mg/l, while the average concentration of nitrate (NO₃-N) was 0.13 mg/l.

A total of 13.71 mg/l of PO₄-P, 12.7 mg/l of COD and 161 mg/l of BOD were found. Figure 3 depicts the mean values obtained from the effluents of five different seafood processing plants. The number of viable plates in the sample is an important metric for assessing the water's quality. Different sampling units had total viable bacterial counts ranging from 1.4107 cfu/ml to 1.0109 cfu/ml.

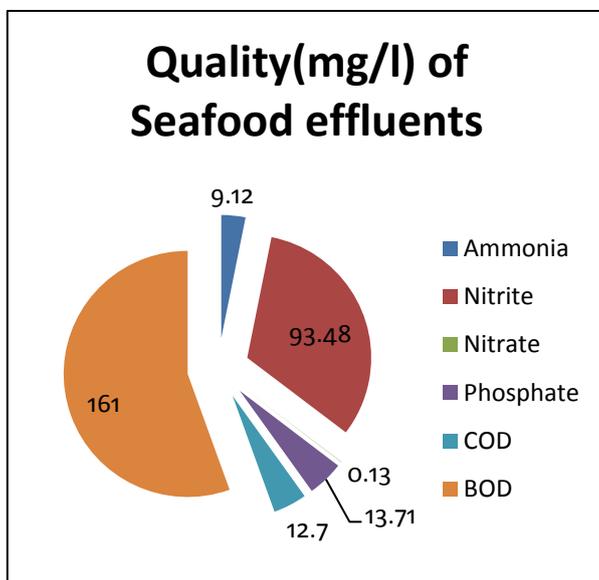


Fig3: Graphical representation of average physico-chemical value (mg/l) of five

Table1: The average physico-chemical value (mg/l) of five

Effluents	Quality(mg/l)
Ammonia	9.12
Nitrite	93.48
Nitrate	0.13
Phosphate	13.71
COD	12.7
BOD	161

Conclusion:

Bioremediation is a promising technology for removing environmental pollutants, restoring contaminated sites, and preventing further pollution. This environmentally friendly technology uses bioconversion, pollution cleanup, and pollutant degradation. Fish processing waste water management is a major environmental problem. The current study used bioremediation to clean up seafood processing waste water. Biodegradation of seafood processing factory effluents utilizing microbes is in the early stages, with a bright potential for bioremediation of waste waters. This study identified bacterial strains for seafood processing plant wastewater bioremediation. This study suggests these microbes can bioremediate seafood plant effluents.

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