

PREPARATION OF A BIOSORBENT IN REMEDIATION OF WATER USING FRUIT PEELS

Submitted in partial fulfilment of the requirements for the award of
Bachelor of Technology degree in Biotechnology

by

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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of V. Bhavani (38230006), G.Sriranjani (38230033) who carried out the project entitled "PREPARATION OF BIOSORBENT IN REMEDIATION OF WATER USING FRUIT PEELS" under our supervision from to

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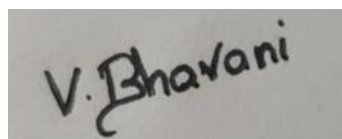
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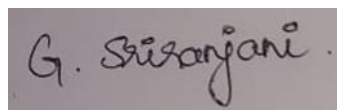
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DECLARATION

We BHAVANI.V (38230006) and SRIRANJANI.G (38230033) hereby declare that the Project Report entitled PREPARATION OF A BIOSORBENT IN REMEDIATION OF WATER USING FRUIT PEELS done by us under the guidance of Dr.D.PRABAVATHY (Internal) and DR.KASINATHAN (External) at ARMATS BIOTEK TRAINING AND RESEARCH INSTITUTE, GUINDY, CHENNAI is submitted in partial fulfillment of the requirements for the award of Bachelor of Technology degree in Biotechnology.

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ABSTRACT

Several researches carried on fruit peels proved that they have a great potential to be used as an adsorbent in water remediation. The purpose of this work is to design a product using fruit peels to remove pollutants from waste water which aids in easy recovery and thus the product can be commercialized. This work is carried out to exploit the removal of pollutants (Ammonia) from aquaculture ponds using orange fruit peel packed in a porous Nylon bag. Though nanoparticle showed to be a solution they are not used commercially because of their cost and unknown effects on marine species. The reason maybe because we lack the knowledge about Nanoparticles effects on Human health on its exposure. To use them without knowing the ill effects on marine ecosystem is not ethically advisable. Fruit peels are natural, abundant, cheaper, ecofriendly and is a good alternative to nanoparticles in water remediation. Fruit peels proved to be a great adsorbent in removing a variety of pollutants. Hence this work is done to investigate the potential of fruit peel in treating aquatic solutions of Aquaculture ponds in ammonia removal. Washed and dried orange peels are powdered to finite size and experimented for different adsorption conditions such as Ph, contact time, adsorption dosage, and initial pollutant concentration. To aid in recovery the powdered peels are packed inside a porous bag and experimented. This work proved there is no significant change in removal of pollutants if the powdered peels are used raw or packed inside a bag. Further the survivability of fish when the product is introduced to them is also tested.

CHAPTER No.	TABLE OF CONTENTS	PAGE No.
	TITLE	
	ABSTRACT LIST OF ABBREVIATIONS LIST OF FIGURES	
1 1.1 1.2 1.3 1.4	INTRODUCTION AQUACULTURE IMPACT OF AMMONIA IN AQUACULTURE NESSLER'S REAGENT KEY ROLE OF ORANGE PEEL	
2	REVIEW OF LITERATURE	
3 3.1 3.2	AIM AND OBJECTIVE AIM OBJECTIVES	
4 4.1 4.2 4.2.1 4.2.2 4.2.3 4.2.4 4.2.5	MATERIALS AND METHODOLOGY MATERIAL REQUIRED METHOD PREPARATION OF ADSORBENT PREPARATION OF ADSORBATE PREPARATION OF PRODUCT ESTIMATION OF AMMONIA CONCENTRATION USING NESLLER REAGENT ADSORPTION EXPERIMENTS	
5 5.1 5.1.1 5.1.2 5.1.3 5.1.4 5.1.5	RESULTS AND DISCUSSION ADSORPTION PARAMETERS EFFECT OF Ph EFFECT OF BIOSORBENT DOSAGE EFFECT OF INITAIAL ADSORBATE CONCENTRATION EFFECT OF CONTACT TIME EFFECT OF TEMPERATURE	

5.2	BIOSORBENT PRODUCT	
5.3	SURVIVABILITY OF FISH	
6	SUMMARY AND CONCLUSION	

LIST OF ABBREVIATIONS AND SYMBOLS:

g – grams

mg – milligrams

μm – micro meter

μl – micro liter

ppm – parts per milli liter

°C – degree Celsius

> - Greater than

< - Lesser than

CaCl₂ - Calcium Chloride

NaOH – Sodium Hydroxide

HCl – Hydrochloric acid

OPP – Orange peel powder

amm – ammonia

Conc- concentration

LIST OF FIGURES

FIG NO	TITLE	PAGE NO
4.2.1	Shade Dry	
4.2.2	Sun Dry	
4.2.3	Desiccator	
4.2.4	Ammonia stock solution	
4.2.5	Porous bag	
4.2.6	Ammonia standard graph	
4.2.7	Adsorption experiment	
4.2.8	Product adsorption test	
5.2	Biosorbent product	
5.3	Survivability of fish	

CHAPTER 1: INTRODUCTON

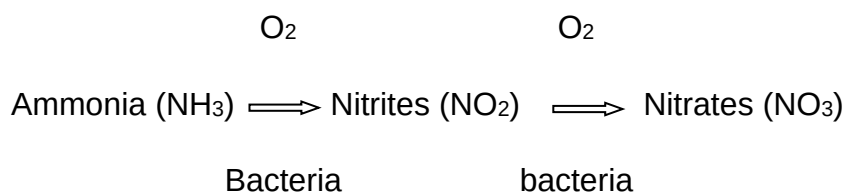
1.1 AQUACULTURE

Aquaculture is the practice of raising aquatic organisms for food. Around 500 BCE, the Romans farmed oysters and fish in Mediterranean lagoons, whereas freshwater aquaculture developed empirically some 1000 years earlier in China. Aquaculture refers to the farming or rearing of fish in confined waters. Aquaculture is taken into account as a double-edged sword because it does not only have tremendous benefits for humans but also great outcomes for the environment. Considering the advantages, seafood produced by fisheries and aquaculture contributes 15 to twenty of the average animal protein consumption to 2.9 billion people worldwide (Martin D. Smith et al,2010) without considering the contribution of freshwater or brackish water species. The nutritional quality of aquatic products features a high standard and represents a crucial source of macro-and micronutrients for people from developing countries (Nanna Roos et al,2007). Additionally, aquaculture and fisheries are recognized as a source of employment; as an example, almost 43.5 million people were employed in 2006, and 520 million people relied on income from seafood production. (Max Troe,2013) Impacts of aquaculture on biodiversity arise from the consumption of resources, such as land (or space), water, seed, and feed, their transformation into products valued by society, and the subsequent release into the environment of greenhouse gases and wastes from uneaten food, fecal, and urinary products, chemotherapeutics as well as microorganisms, parasites, and feral animals. Aquafarming is the distribution of aquatic organisms under controlled, semi-controlled, or simulated conditions. While the fish are kept in a controlled environment, the utilisation of ponds and recirculation systems adds a new level of difficulty, especially in the closed nature of the cultures. Though there is a growing demand for aquaculture, we face a major drawback which is the influence of ammonia on fish behaviour and health. Exposure to toxic substances in the aquatic environment can induce negative effects on reproduction and growth performance in fish (Kim and Kang 2015). Among various toxicants, ammonia is one of the foremost toxic substances to cause the growth inhibition in fish farming, and therefore ammonia toxicity is often the main reason for fish mortality (El-Shafai et al,2004). Given that the toxicants generally inhibit the growth performance in aquatic

animals, the growth performance is often an honest indicator to assess the toxicity within the animals.

1.2 IMPACT OF AMMONIA IN AQUACULTURE

Ammonia is a compound present in water at pH<9. Ammonium has no toxic effects on human health, but its presence in water can indicate pollution (Radu G and Racoviteanu G,2021). Ammonia is one of the nitrogenous wastes, especially in water. it's generated from the catabolism of amino acids, purines, and pyrimidines (Ruyet et al. 1995). Ammonia plays an important part in the action of any submarine terrain (Chris sergeant,2014). Excessive ammonia can cause the growth performance to decrease, tissue erosion and degeneration, immune suppression, and high mortality in aquatic animals, which act as toxicity by increasing ammonia levels in blood and tissues (Lemarie et al. 2004; Li et al. 2014). (LaDon Swann ,1990) Two sorts of ammonia occur in the aquaculture systems Ionised and un-ionised. Toxicity levels of unionized ammonia rely on individual species. However, levels below 0.02 ppm are considered safe. (Chris sergeant,2014) The oxidative process during which ammonia is first converted into nitrite (NO₂) by naturally being Nitrosospira and Nitrosomonas bacteria within the water, before further bacterial species Nitrospira and Nitrobacter convert the nitrite into nitrate (NO₃). The un-ionised variety of ammonia is extremely toxic to fish while ionized ammonia isn't. Both forms are grouped together as “total ammonia.” Through biological processes, toxic ammonia is often degraded to harmless nitrates (LaDon Swann, 1992).



This nitrification process occurs moreover on the slush substrate and within the biofilter of a tank grounded system. The nitrite continues to be poisonous to fish species but increases the growth and settlement of Nitrobacter to convert it to the lower poisonous nitrate form. The nitrate is also taken up by submarine and algae in monoculture systems. The natural process is directly affected by oxygen and alkalinity situations, with a reduction in either terminating the cycle and therefore adding ammonia and nitrite concentrations over again. it's worth consider that the

ammonia concentration favours the growth of specific phytoplankton and algal species, which alters the submarine biodiversity and dynamics of an ecosystem (Chris sergeant,2014).

In essence, nitrification is the oxidation of nitrogen compounds (the transfer of electrons from the nitrogen atom to the oxygen atoms (©2010 YSI Inc). Understanding how nitrification rates are affected by a number of things is crucial and taking ammonia readings at different times will give you a lot of information. For clear management decisions, a greater understanding is required for Ammonia, dissolved oxygen concentration and temperature, all of them play a significant role. Thus, the concentration of ammonia is lower in the summer. so, that nitrification rates are often low. With the help of bacterial communities, excess ammonia can be handled. During the colder months, Microbial activity is suppressed at low temperatures. Temperature and ammonia concentrations are higher in the spring and fall, which favours in increasing nitrification rates. During, the seasons of spring and autumn in many fish ponds, there are common peaks in nitrite concentrations (©2010 YSI Inc).

Ammonia enters the submarine terrain primarily from the fish themselves via their waste. Fish diet will frequently contain high saturation of protein, which when metabolised, produces ammonia as a by- product. The fish also excretes the ammonia from its blood into the water via a partial pressure grade across its gill epithelium, also as a small quantity within the urine. The salutary protein level within the feed is directly proportional to the ammonia concentrations produced with more protein levels give rise to further ammonia. Other sources can include the spoilage of algae or uneaten food within the submarine terrain, with lower volumes of water more at threat of ammonia spikes (Chris sergeant,2014).

Ammonia 'sinks' takes place within pond monoculture within the specific type of the plants and algae. These organisms need nitrogen as a crucial nutrient for growth, and as a result it absorbs ammonia from the water. As a rate of photosynthesis increases, plant and algal growth increases and also the uptake of ammonia increases. While the presence of algae within a monoculture system does directly influence the reduction in ammonia concentration, it's not a feasible long- term system for ammonia removal. During the downtime months, ammonia product is

harmonious but algal ammonia uptake is confined. This causes a rise in ammonia concentration, and accordingly fish may become stressed-out at a period of time during which the immune system of a fish is suppressed because of lower temperatures (Chris sergeant,2014).

1.3 NESSLER'S REAGENT

Nessler's reagent is used to detect ammonia. It's a pale solution which gets darker yellow when some volume of ammonia is added. A brown precipitate may form at high concentration. Nessler's reagent is a result containing K_2HgI_4 and KOH. Iodide and mercury ions react with ammonia under alkaline conditions to produce a yellow - brown complex, which absorbs strongly at 420 nm.

1.4 THE KEY ROLE OF ORANGE PEELS

The food sector is known for producing a large amount of waste and by-products. Despite the negative effects on the environment, the volume of agro-industrial waste has continued to rise. Orange peels are a good example of agro-industrial waste, as they are the by-product of fruit processing for juice manufacturing. Because of the vast volume of garbage generated and the environmental risk it poses, some research has focused on valuing it through the extraction of value-added goods or even the transformation of waste into other materials.

Valorisation of trash is an appealing concept that offers potentially valuable alternatives to disposal or landfill deposition for waste treatment. In general, the characteristics of agro-industrial waste vary depending on the raw material and the procedure that produced the residues; nonetheless. Biosorption is the simple yet effective method for removing ammonia from water.

Biosorption is a natural physiochemical mechanism that allows specific biomass to passively concentrate and bind pollutants to its cellular structure. The synthesis and characterization of low-cost, organic, biodegradable, anionic magnetic sorbents derived from natural waste material (orange peel) to remediate organic pollutants from water. The orange peels bio sorbent is one of the best and cost-effective bio sorbents for removal of ammonia present in water.

CHAPTER 2: REVIEW OF LITERATURE

You may have heard something about the aquarium cycle and waiting for ammonia and nitrite to reach zero before adding fish. Although it may appear odd at first, ammonia is an important biological element of aquatic systems that must be handled under certain way. Fortunately, microorganisms do the majority of the work, but sometimes a water change or chemical conditioners are required (Stuart M. Levit, 2010).

Ammonia can change chemical form/ species which changes its impacts and behaviours, similar as toxin, availability, etc. On the surface ammonia may change form, such as to nitrates or nitrites but is less likely to change forms in groundwater and thus ammonia itself is more likely to live in groundwater (Morin 2009; Forsyth 1995). Ammonia can be produced naturally from the breakdown of organic matter and is excreted by fish as a nitrogenous waste product. In fish, ammonia is a by-product of protein metabolism and is primarily excreted across the gill membranes, with a small quantity excreted in the urine. Ammonia produced by fish can be excluded by bacterial conversion of ammonia to nitrite and nitrate. Nitrate can be used by plants and algae and is generally considered harmless to fish in natural waters (Francis-Floyd 2009). Ammonia's submarine toxin is basically due to the unionized form, NH_3 (Arthur et al. 1987). As pH increases, the toxicity of ammonia increases because the relative proportion of unionized ammonia increases (Brinkman et al. 2009; Paley et al. 1993; EPA 1999).

Ammonia and Fish

Ammonia is a poisonous compound that can harm fish health. Many factors impact the nature and degree of toxin, including the chemical form of ammonia, the pH and temperature of the water, the length of exposure, and the life stage of the exposed fish. Issues associated with toxicity testing and the consequences of ammonia exposure.

Sources in aquaculture

(Claude E. Boyd,) Diseases and feeds are the most current sources of ammonia in monoculture ponds, and issues with high ammonia are most common in feed-based

monoculture. Ammonia is expelled by culture creatures as a waste product of protein metabolism, and it's also produced by microbial breakdown of organic substances. As feed input increases in response to increased product, ammonia concentrations tend to rise.

In ponds, there are a many natural limitations on the overall ammonia nitrogen absorption. The two main ones are uptake of ammonium by phytoplankton and oxidation of ammonia nitrogen to nitrate by nitrifying bacteria. When the pH of a pond is raised, ammonia can diffuse into the air, and diffusion is a slightest loss of ammonia from ponds on windy days and in heavily aerated ponds. Of course, outflowing water removes ammonia, and small quantities of ammonium may be adsorbed on cation exchange sites in bottommost soil (ClaudeE. Boyd.).

Ammonia Speciation, Temperature, and pH

When dissolved in surface water, ammonia exists in two forms NH_3 (unionized) and NH_4 (ionized). Ionized ammonia doesn't freely cross fish gills and is less bio available than the unionized form (Francis-Floyd 2009; EPA 1989). The unionized form can cross from water into fish, and formerly outside, some converts to the ionized form which also causes cellular damage (EPA 1989). The primary form of total body ammonia in fish at physiological pH (7.0 –8.0) is ammonium, or NH_4 ; it's this chemical species that's responsible for poisonous goods (NH_3 is the most poisonous form to submarine life, but NH_4 is the most poisonous form in the body) (McKenzie et al. 2008; Smart 1976; Hillaby and Randall 1979). Although unionized ammonia is the more poisonous form, toxicity is most generally expressed as total ammonia-the sum of NH_3 and NH_4 in water. Ammonia is more poisonous to submarine life at high temperature and pH values. As pH increases, so does the small amount of unionized ammonia and the toxicity to fish (EPA 1999). The rate of NH_3 to NH_4 increases by 10 times for each one-unit rise in pH, and by roughly 2 times for each 100 C rise in temperature from 0-300 C (US EPA, 2009).

Ammonia toxicity is also a function of ionic strength, or the saltiness of the water. Several studies have indicated that adding the hardness of ambient water can in some cases drop ammonia toxicity (Wicks et al. 2002; Soderberg and Meade, 1992). In general, seawater species are slightly more sensitive to ammonia toxin than brackish species (Randall et al. 2002).

It makes sense that the unionized form would be more poisonous. Unionized ammonia is a neutral patch and can diffuse across cell membranes of submarine organisms more fluently than the ionized form (EPA 1999). As formerly described, ammonia is naturally produced by fish and their strategies to excrete the unionized form are largely dependent on unresistant diffusion across the gills-which would be much less effective or insolvable in waters with high external unionized ammonia. In other words-water with advanced unionized ammonia will reduce or reverse the diffusion grade, causing fish to condense ammonia in their gills and blood (EPA 1999).

The concentrations of ammonia that bring toxicity to fish depend on the length of exposure. Lower concentrations may not kill or negatively affect fish over short periods of time, but the same concentrations could kill or impair submarine life under longer time frames. US EPA recommends an acute ammonia criterion of 2.9 or 5.0 mg N/ L (for short- term exposure) and a habitual criterion of 0.26 or 1.8 mg N/ L (for long- term exposure). Water with concentrations of lower than 0.020 mg/ L unionized ammonia is considered safe for fish reproduction (EPA 1989). Lower and lower absorption of ammonia are permitted with adding pH values under acute (short-term) exposure scenarios. The same is true under longer term exposures, and lower concentrations are permitted under advanced temperatures.

A single or simple concentration dimension of ammonia isn't sufficient to estimate and/ or regulate ammonia impurity. The influence on the toxicity of ammonia to fish is impacted by both duration and frequency of exposure. Duration of exposure to ammonia has been demonstrated to be a critical factor affecting fish survival and should thus be incorporated into habitual and acute norms. The demonstrated influence of exposure frequency indicates the need to consider the return period of transient pollution events in inferring applicable standards because the return period will basically impact the degree of recovery following exposure and, accordingly, the long- term viability of fish populations (Milne et al. 2000).

Consequences of Ammonia Exposure

Fish are exposed when ammonia enters a fish across its gill epithelium - in freshwater this may be almost exclusively via passive diffusion of ammonia as a gas in solution (McKenzie et al. 2008; Randall and Wright 1987; Ip et al. 2001; Randall

and Tsui 2002; and Eddy 2005). Ammonia is also produced as an end product of metabolism and then excreted across the gill epithelium (McKenzie et al. 2008; Randall and Wright 1987; Wright 1995; Randall and Tsui 2002). Ammonia can accumulate in fish as a consequence of exposure to elevated water ammonia concentrations or when excretion of the endogenous metabolite is inhibited (McKenzie et al. 2008).

Ammonia can be acutely toxic to fish mainly due to its effect on the central nervous system, because it causes “acute ammonia intoxication” - which includes convulsions and death (Randall et al. 2002). Concentrations of ammonia that are acutely toxic to fish may cause loss of equilibrium, hyperexcitability, increased breathing, cardiac output, and oxygen uptake, and, in extreme cases, convulsions, coma, and death (EPA 1989). Lower concentrations of ammonia can cause a reduction in hatching success, reduction in growth rate and morphological development, and pathologic changes in tissues of gills, livers, and kidneys (EPA 1989).

The majority of fish species cannot withstand high levels of total ammonia (Randall et al. 2002). Elevated ammonia levels in the environment can inhibit ammonia excretion or cause a net intake of ammonia from the environment in fish, resulting in elevated ammonia levels in the body, which can eventually lead to convulsions and death (Randall et al. 2002).

Escape responses is a reflex that comprises a sudden and brief acceleration, typically in a direction away from the startling stimulus, such as a predator, and are used by fish as a defence mechanism (McKenzie et al. 2008; Domenici and Blake 1993). The success of a predator avoidance escape response is determined by both sensory and locomotory performance (McKenzie et al. 2008). Temperature, dissolved oxygen, and turbidity are all factors that influence the escape reaction (McKenzie et al. 2008).

Some fish have been shown to have impaired startle-escape response performance in response to sublethal ammonia concentrations, and ammonia accumulations in the water and in mullet tissues impaired various sensory and motor components of the response, often in a manner that appeared to be proportional to the ammonia concentrations. (McKenzie and et al, 2008).

Responsiveness (an indicator of acoustic or visual sensitivity and incentive to flee) and directionality (an indicator of overall sensory performance) were the only two performance characteristics not influenced by ammonia in that study (McKenzie et al. 2008).

Ammonia can affect a fish's central nervous system, causing hyperventilation, hyperexcitability, and loss of equilibrium (as well as convulsions, coma, and death, as described elsewhere) (McKenzie et al. 2008; Olson and Fromm 1971; Hillaby and Randall 1979; Arillo et al. 1981; McKenzie et al. 1993;). Sublethal ammonia concentrations in rainbow trout (*Oncorhynchus mykiss*) can generate hyperexcitability, resulting in fish rushing into the tank's sides in response to any disturbance (McKenzie et al. 2008; Olson and Fromm 1971). Salmonids' ability to perform exercise in an incremental "critical swimming speed" protocol can be impaired by sublethal ammonia accumulations in plasma (hyperammonemia), which can mean that fish are unable to escape predation or other threats because they are unable to recruit white muscle to power anaerobic burst swimming at relevant speeds (McKenzie et al. 2008; Beaumont et al. 1995; Shingles et al. 2001; Wicks et al. 2002). Increased delay of the response and poorer kinematic performance would thus put the fish at higher danger of predation from predators such as birds and mammals that would not accumulate or be similarly impaired by ammonia toxicity (McKenzie et al. 2008).

Conclusion

Biosorption is a natural physiochemical mechanism that allows specific biomass to passively concentrate and bind pollutants to its cellular structure. It is a metabolically passive process that does not require energy, and the number of pollutants that can be removed from the sorbent is determined by kinetic equilibrium and the composition of the sorbents at the cellular surface. Every bio sorbent had different physical, chemical and biological properties for heavy metals removal by biosorption from the water.

For removing ammonia from contaminated water, biosorption is a simple, cost-effective, and environmentally acceptable approach. one yet solution for ammonia removal is, orange peels. They were employed as locally accessible bio sorbents (with and without chemical treatment) for ammonia removal via biosorption

processes. Because of their high surface areas and active functional groups, orange peels are widely utilised as adsorbents in wastewater treatment, although their high cost occasionally prevents their utilisation.

CHAPTER 3: AIM AND SCOPE

3.1 AIM

To prepare a natural biosorbent product in removal of pollutants from aqueous solution and to commercialize the designed product for daily use.

3.2 OBJECTIVES

- To prepare a biosorbent from fruit peels to be used in water remediation.
- To design a product where the powdered peels are packed in a porous bag to aid in easy recovery and reuse.
- To demonstrate the use of designed product in removal of pollutant from aqueous solution.
- To make a commercially available ecofriendly adsorbent.

CHAPER 4: MATERIALS AND METHODS

4.1 MATERIAL REQUIRED

- Orange fruit peel
- Nylon cloth
- Electric grinder
- Sieve mesh (500 μ m and 250 μ m)
- Ammonia chloride salt
- Distilled water
- Nessler reagent
- Spectrophotometer
- Test tubes
- Conical flasks
- pH meter
- Rotatory shaker
- Weighing scale
- Desiccator
- Incubator
- Pipette
- Volumetric flask
- Filter paper

4.2 METHODS

4.2.1 PREPARATION OF ADSORBENT

Orange fruit peels were collected from fruit shops and were washed with distilled water. Washed fruit peels were divided equally in weight for to sundry and shade dry. In sundry, peels were directly kept under the scorching sun for 2 days. In shade dry, peels were kept in laboratory at room temperature for 1 day and incubated at 40°C for next 24 hours. Dried fruit peels after sundry and shade dry were powdered separately using an electric grinder (mixer) into fine particles. Sun dried powdered peels were sieved using sieve meshes of 500 μ m and 250 μ m sizes to obtain different particle sizes of biosorbent. Obtained sizes are noted as >500 μ m, between 500 μ m and 250 μ m and <250 μ m respectively. Powdered peels of size between 500 μ m and 250 μ m is used for biosorption experiments. The powdered peels were stored in desiccator when not in use for longer shelf life. Desiccator is used to remove moisture by using anhydrous calcium chloride (CaCl₂) as drying agent. Visual observation of powdered peels from both sundry and shade dry was of no significant difference. Henceforth sundried powdered peels were chosen for adsorption studies.



Fig 4.2.1 Shade dry

a -orange; b -washed peel; c - shade dried for a day; d - incubated for 24h; e - powder

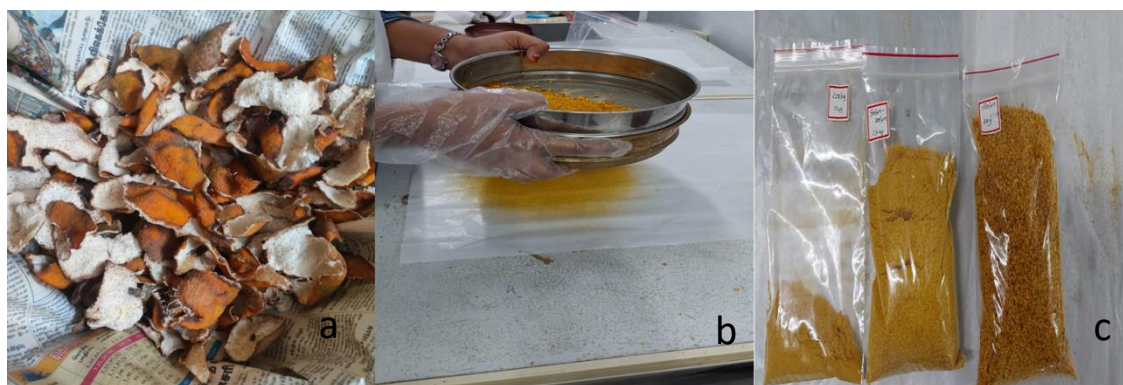


Fig 4.2.2 Sun dry

a – sundried for 2 days; b – sieve meshes; c – different particle size

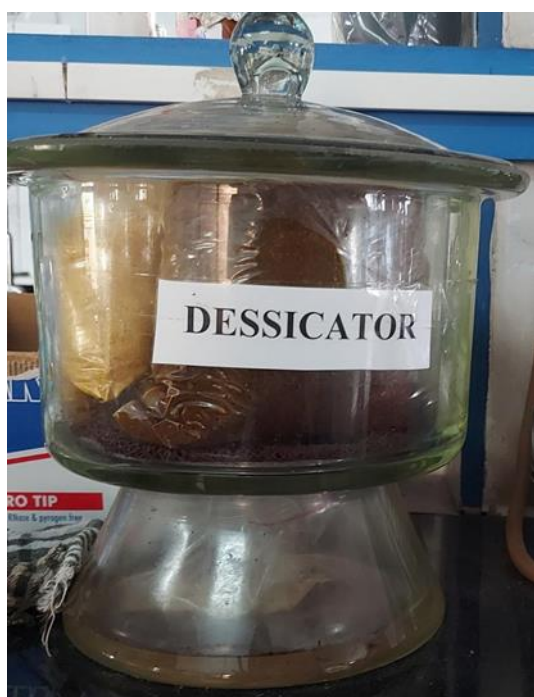


Fig 4.2.3 Desiccator

4.2.2 PREPARATION OF ADSORBATE

Stock solution of ammonia was prepared by dissolving 10mg of ammonium chloride salt in 100ml of distilled water. Thusly 100ppm of ammonia stock solution was made. Working standard were made from diluting stock solution.



Fig 4.2.4 ammonia stock solution

4.2.3 PREPARATION OF PRODUCT

OPP of 1g was packed inside a nylon cloth with its opening sealed. The particle size of OPP exceeds the pore size ensuring there is no escaping of OPP. The prepared porous bag allows OPP to access the water and aids in adsorption process. The particle size of OPP packed inside is between 500 μ m and 250 μ m.

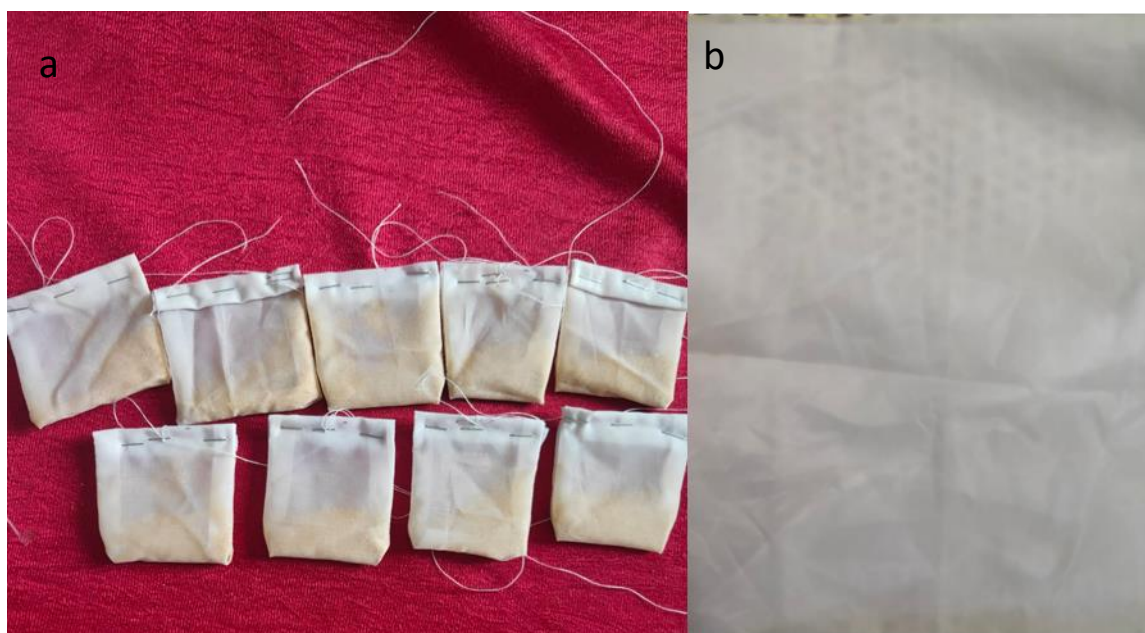


Fig 4.2.5 Porous bag

a – packed product; b – nylon cloth

4.2.4 ESTIMATION OF AMMONIA CONCENTRATION USING NESLLER REAGENT

Standard graph was plotted for different concentrations ammonia in ppm against absorbance. The graph was prepared to estimate the unknown ammonia concentration in the aqueous solution. Concentrations of ammonia ranging from 1ppm – 10 ppm were prepared by diluting 0.1ml, 0.2ml, ..., 1ml from stock solution in 9.9ml, 9.8ml, ..., 9ml of distilled water respectively in different test tubes. To each test tube 125 μ l of Nessler reagent was added. Ammonia in reaction with Nessler reagent produces yellow brownish colour by process of nesslerization. Readings were taken at 425nm using spectrophotometer. Graph was plotted with ammonia concentration as X-axis and absorbance as y-axis.

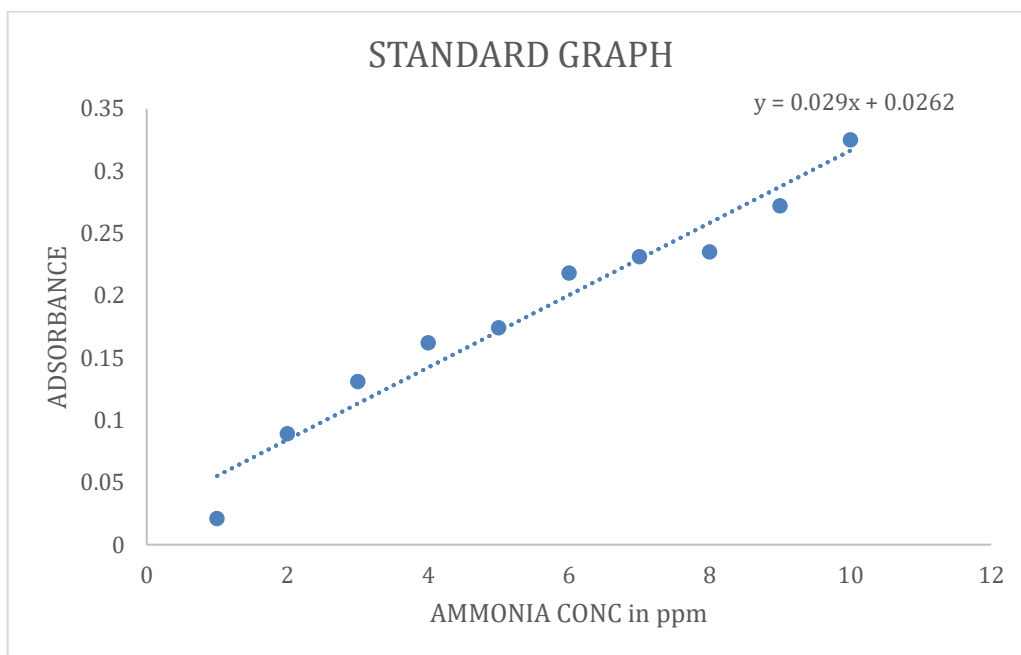


Fig 4.2.6 Ammonia standard graph

4.2.5 ADSORPTION STUDIES

The adsorption of ammonia by orange peel was studied by batch method. Known volume of ammonia solution with varying initial concentration was mixed with fixed

dosage of adsorbent at specified contact time in 5 different test tubes with occasional shaking. The solution was then filtered with Whatman filter papers. The filtrate was collected in new test tubes. 2ml of solution from each test tubes were carefully pipetted out into different test tubes. To test tubes containing 2ml of filtrate, 25 μ l of Nessler reagent were added. Upon colour formation, readings were taken at 425nm using spectrophotometer. The OD obtained was plotted in standard graph to estimate the removed concentration of ammonia by the orange peel powder. This proved ammonia is efficiently removed by OPP.



Fig 4.2.7 Adsorption experiment

a – known vol of amm; b – OPP added; c – filtration; d - nesslerization

bpowder. By taking known volume of ammonia solution with varying initial concentrations and constant 1g of OPP product at specified contact time in 250ml conical flask with occasional shaking was tested for adsorption. 2ml of solution was pipetted into clean test tubes to which 25 μ l of Nessler reagent was added. Upon colour formation, readings were taken at 425nm using spectrophotometer. The OD obtained was plotted in standard graph to estimate the removed concentration of ammonia by the orange peel powder. 1g of OPP removed 5ppm of ammonia completely.

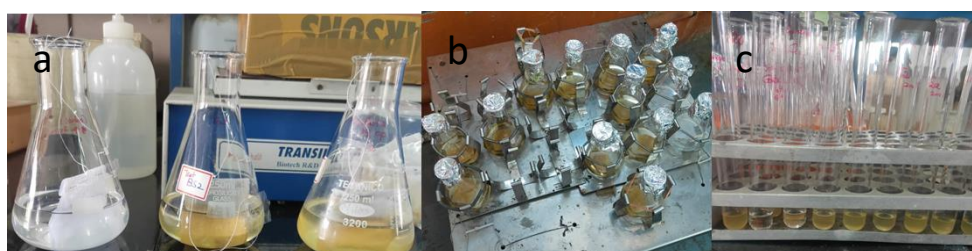


Fig 4.2.8 Product adsorption test

a – control and test; b- 1g OPP and varying amm conc; c- nesslerization

With both the ammonia concentration and OPP dose as constant, adsorption was checked for varying pH of 4.5, 5.5, 6.5, 7.5, 8.5, 9.5 respectively. pH was adjusted by using NaOH for basic and HCl for acidic condition. Adsorption of ammonia was favored in basic conditions.

With varying contact time from 15min, 30min, 45min, 60min and 24h and keeping ammonia concentration and OPP dose as constant, adsorption was tested. Adsorption took place within the 15min and slowed down upon reaching equilibrium at 1h. All the adsorption experiments were done on room temperature.

5. RESULTS AND DISCUSSION

5.1 ADSORPTION PARAMETERS

5.1.1 Effect of pH

pH effects biosorption. Aquaculture ponds maintain a certain particular pH which is the optimum condition for fish growth. It is said that ammonia even at very lower concentrations at alkaline conditions is toxic to fish. In acidic conditions ammonia is not toxic. So it is crucial to check adsorption at different pH conditions.

5.1.2 Effect of Biosorbent dosage

It determines the saturation point where there will be no significant increase in adsorption. An increase in biosorbent increases adsorption as there is an availability of more vacant binding sites. More vacant sites will make it easier for the adsorbates to bind. Further increase in biosorbent after saturation will not improve adsorption process (Maksoud, M. A., et.al ,2020).

5.1.3 Effect of initial adsorbate

Increase in initial concentration of pollutant enhances the biosorption capacity of adsorbents but decreases the rate of adsorption (Maksoud, M. A., et.al ,2020).

5.1.4 Effect of Contact time

Contact time is an important matter because it is essential to give sufficient amount of time for the adsorbent and adsorbate to interact. Adsorption rate is increased when there are more binding sites available for the adsorbate to bind and as it gets occupied over time there will be a gradual decrease in the adsorption rate since binding sites are getting filled (Maksoud, M. A., et.al ,2020).

5.1.5 effect of Temperature

Fishes or any other marine species that which is cultivated in ponds cannot tolerate higher temperatures. Therefore, it is important to check whether adsorption happens at the temperature in which the fishes are grown. Ammonia adsorption by OPP happens at room temperature.

5.2 BIOSORBENT PRODUCT

Fruit peels role as a natural biosorbent was studied and various researches were conducted on their ability to remove a variety of pollutants from aqueous solutions. Though their significance was recognized they are not commercially used in water treatments. Hindering their day to day is the recovery and way to incorporate them in real life application. OPP are solids with a tendency to settle down on bottoms. If the particle are lighter they float in the water. When OPP is introduced directly into the aquaculture ponds or any other water bodies with no water flow, the solid particles settle at the bottom. Theoretically speaking there is no valid evidence that the raw OPP removed the pollutant and it is nearly impossible to recover the used OPP. In order to overcome this problem, we need a way where the OPP can be easily introduced into a water body and can be recovered simply. In laboratory adsorption test are carried out with agitation to ensure the contact between adsorbent and adsorbate. The proposed product is made with a porous bag which allows water to enter and leave thereby allowing interaction between the OPP inside the bag and water outside the bag. Besides the porous bag floats in the water which drifts on its own ensuring minimal agitation. The product is easy to use and simple to recover. In case of raw OPP, recovery is done by filtration or centrifugation. This is helpful in recovery and reuse of OPP. On comparison with raw used OPP and porous bag packed OPP in adsorption, they showed similar results. Thusly the product made can find its application in ammonia removal from aqueous solutions.

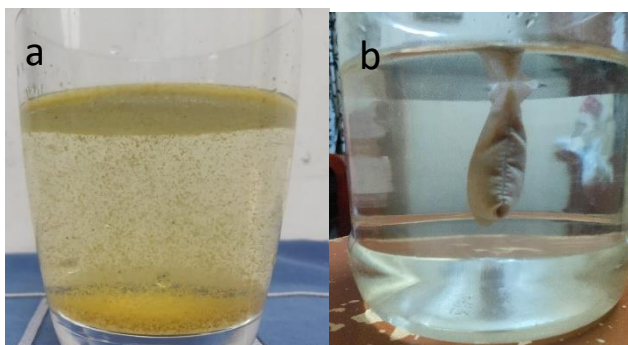


Fig 5.2 Biosorbent product

a – raw OPP b- Product

5.3 SURVIVABILITY OF FISH

The aim of this work is to prepare a product to help in water remediation and to commercialize it. The product was designed to find its application in aquaculture for ammonia removal from the aqueous solution. Two tanks were set up with normal tap water and the pairs of fishes were introduced to them. The tanks were left untouched for 2 days to make sure the fishes adapt to their new environment. On the next day, product was introduced in 1 of the fish tanks and monitored closely for visible changes in fishes. After an hour it can be observed that there is sharp change in their movements. Their movement were slowed and they remained motionless showing signs of distress. Regrettably the fishes could not survive for more than 5 hours in OPP introduced water. *Barbus tetrazona* commonly called as Tiger barb was the species used for this test. The tiger barb is said to tolerate acidic condition (pH-6.5) and OPP induces acidic condition. Yet the fishes could not tolerate OPP introduced environment and the cause for this cannot be reasoned by this work because of lack of knowledge.

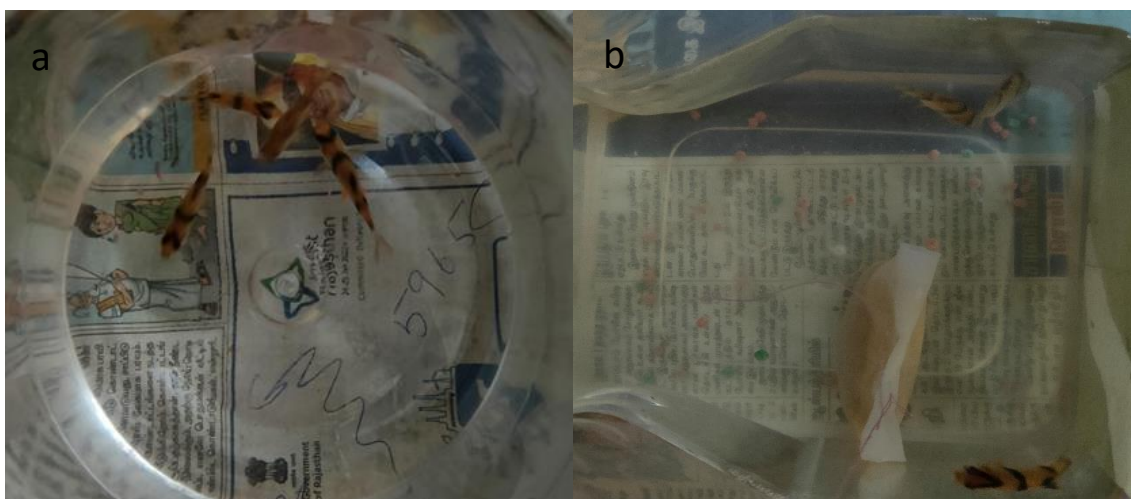


Fig 5.3 Survivability of fish

a – Tank1 b – Tank 2 with product

6 SUMMARY AND CONCLUSION

Orange fruit peel was used in removal of ammonia from aqueous solutions by the process of adsorption. Adsorption is a simple process wherein sorbate molecules accumulate at sorbent surface. Adsorbate refers to the substance that is adsorbed and Adsorbent is a solid substance that can adsorb other substances. This work focuses on making a product which can find its application in aquaculture ponds in removal of ammonia by the process of adsorption. Orange peel was collected from households and local fruit shops and washed with distilled water. Washed peels were subjected to shade dry and sundry separately. Dried peels were powdered using electric grinder, sieved using sieve meshes to obtain different particle size and stored in a desiccator to ensure long term use. Stock solution of ammonia was prepared by dissolving ammonium chloride in distilled water. Working standards were by diluting stock solution. Nessler reagent was used to produce yellow brownish colour in reaction with ammonia during nesslerization process. The colour intensity produced is directly proportional to ammonia concentration. Spectrophotometer readings were taken at 425nm. Product was prepared by packing the OPP inside a porous nylon cloth to make recovery easy. Adsorption was experimented by checking parameters such as pH, contact time, adsorbent dosage, initial pollutant concentration and temperature. The experiments were repeated for atleast 3 times for approximate values and results. Adsorption happened at alkaline conditions and at room temperature. On comparison with raw OPP and product in adsorption efficiency, they showed similar results of adsorption. Using raw OPP will make the recovery tough because it can be done only by filtration or centrifugation. In application, filtration of a huge aquaculture pond is not feasible. Hence the product was designed to aid in recovery and reuse of OPP. Product ensures efficient adsorption, simple to use, easy to recover. In

addition to adsorption, survivability of fish in OPP introduced environment was also tested. Species *Barbus tetrazona* was monitored for noticeable changes in their habitat. Regrettably fishes could not survive OPP introduced environment and they die within 5 hours of contact with OPP. Product designed was promising in their use in aquaculture ponds for their regular ammonia removal. Product prevents escape of packed OPP inside it with allowing them to contact the outside pollutant. Product floats and drifts when introduced in water.

This work concludes that OPP packed in a porous bag can be used a biosorbent in removal of ammonia from aqueous solution. Adsorption is a simple process but the laboratory experiments are required to be repeated for atleast 3 times for accurate results. Product was tested in aquaculture ponds condition and results proved adsorption happens at alkaline condition at room temperature. The result of the experiments proved the orange fruit has greater ammonia removal when used directly or packed inside a porous bag. The product finds its promising use in water remediation.

Outcome: product designed was easy to use, recover and can be applied in water remediation. Preparation of adsorbent is simple and cheap. Preparation of product doesn't require technical skills. Product can be used simply without any technical requirements. The porous bag works similar as a tea bag in a cup of milk. Easy to use, simple to recover and does not require specialized skills are the conditions the product met and tested for the same purpose.

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