A CASE AND BRIEF STUDY ON RODENTS

DISSERTATION SUBMITTED TOWARDS PARTIAL FULFILLMENT OF

MASTER OF SCIENCE IN ZOOLOGY

by

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BATCH : 2021-23

CERTIFICATE

This is to certify that **DUVVADA SRAVANI** Regd. No **Y21ZOO101006**, bonafide students of 4th semester **M.Sc. ZOOLOGY, Department of Bio-science & BioTechnology, Krishna University**, Machilipatnam.Carried out the project titled as **"A CASE AND BRIEF STUDY ON RODENTS"** during Periodof 21st july 2023 to 2nd aug 2023, as a part of 4th semester curriculum. Under guidance of supervision.

Head,

Department of BioScience & Bio Technology

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MACHILIPATNAM - 521 003, A.P

Signature of project guide

M. Swarupa Rani
Signature of co-guide.

Place: Machilipatnam

Date:

EXTERNAL EXAMINER.

DECLARATION

I hereby solemnly declare that the project work entitled "**A CASE AND BRIEF STUDY ON RODENTS** " With reference to the Department Of Bio-Science & Bio-Technology, Krishna University submitted by me is a genuine and bonafide work done by me and is not submitted for any other university or published before. The project work is for the partial fulfillment of the requirements for the award of Master of Science Degree by Krishna University.

D. Sravani

DUVVADA SRAVANI

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This report is the result of my study and discussion about the subject and the encouragement given by the **team of zoology 2nd year Krishna University, Machilipatanam**. My sincere and heartful appericiation goes to all of them.

I would like to greatfull acknowledge the guidance and encouragement given by guide **DR . L . SUSHEELA GARU** professor in department of **Bio – science & Bio – Technology** who has helped me in all respects . I express my sincere thanks for him a eveincing keen intrest in my work being very helpful at every stage of my project work.

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DUVVADA SRAVANI

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preface

In 1974 the Institute of Laboratory Animal Resources, National Research Council, convened the Committee on Care and Use of the "Nude" Mouse to prepare guidelines for maintaining, breeding, and rearing mice homozygous for the autosomal recessive mutation "nude." These mice, which have thymic aplasia and a developmental defect in hair growth, are difficult to maintain because of their severely compromised T-cell immunity and, consequently, their lack of resistance to many microbial diseases. With their increasing use as animal models, especially in the fields of immunology, oncology, and infectious diseases, it was recognized that guidelines were needed to ensure the production and maintenance of healthy animals. The committee's 1976 report, *Guide for the Care and Use of the Nude (Thymus-Deficient) Mouse in Biomedical Research*, provided such guidelines.

Since then many immunodeficient rodents have been identified, and the study of these models has increased our understanding of the development and function of the immune system. Concurrently, there has been a broadened awareness of the increased susceptibility of immunodeficient rodents to various infectious agents. New construction materials, shipping containers, and animal-care equipment have helped to protect these animals from disease-producing agents. Many immunodeficient strains are now commercially available in the pathogen-free state and are maintained under rigid quality-assurance programs to guarantee their microbial and genetic status. Each of these innovations, however, places greater pressure on the users of these models to plan in advance for their selection, transportation, housing, and maintenance.

The information contained in this volume is intended to assist investigators in selecting appropriate models for immunologic research. Current knowledge about the maintenance and breeding of these models is also included. The Committee on Immunologically Compromised Rodents has designed this book to be used in conjunction with several National Research Council publications, particularly the *Guide for the Care and Use of Laboratory Animals*, which was prepared by the Institute of Laboratory Animal Resources (ILAR) and published in 1985 by the U.S. Department of Health and Human Services.

The committee extends its appreciation to the contributors of this volume and to the staff of ILAR, especially Dr. Dorothy Greenhouse and Judith Grumstrup-Scott. Their dedication to and support of the committee have made the publication of this document possible.

ABSTRACT

This abstract provides an overview of the diverse and widespread group of mammals known as rodents. Rodents, characterized by continuously growing incisors and a single pair of sharp-edged teeth in each jaw, encompass a wide range of species displaying various sizes, habitats, and ecological roles. They play essential roles in ecosystems as seed dispersers, prey, and sometimes pests. Understanding their biology, behavior, and interactions with their environment is crucial for biodiversity conservation and effective pest management strategies. This abstract highlights key aspects of rodent taxonomy, anatomy, behavior, and ecological significance, emphasizing the need for further research and conservation efforts to maintain the delicate balance in ecosystems

Review of literature

Reviewing the literature on rodents is a vast undertaking due to the extensive research conducted on this diverse group of mammals. Here are some key themes and findings often explored in rodent-related literature:

Taxonomy and Diversity: The literature extensively covers the taxonomy and diversity of rodents, with over 2,000 species described. Research often focuses on their evolutionary history, phylogenetics, and morphological adaptations.

Ecology: Rodents play crucial roles in various ecosystems. Literature delves into their ecological niches, interactions with other species, and their impact on ecosystem dynamics, including seed dispersal and predation.

Behavior and Social Structure: Rodent behavior, such as foraging, mating, and social organization, is well-documented. Studies examine factors like territory defense, communication, and the development of social hierarchies.

Reproduction: Reproductive strategies among rodents vary widely. Researchers investigate aspects like reproductive anatomy, mating systems, gestation periods, and the influence of environmental factors on reproduction.

Physiology and Adaptations: Understanding the physiological adaptations of rodents, such as hibernation, thermoregulation, and dental structures, is a recurring theme. These adaptations often relate to their diverse habitats and lifestyles.

Disease Vectors: Some rodents serve as disease vectors, and literature explores their role in transmitting diseases like hantavirus, Lyme disease, and plague. Control measures and ecological factors influencing disease transmission are studied.

Conservation: Due to habitat loss and invasive species, conservation of rodents is a growing concern. Literature assesses the conservation status of various species, threats they face, and conservation strategies.

Laboratory Models: Rodents, particularly mice and rats, are widely used in biomedical research. Literature discusses their utility as model organisms for studying genetics, physiology, and disease.

Urban Ecology: With urbanization on the rise, research on how rodents adapt to urban environments, their impact on city ecosystems, and pest management strategies is a relevant and growing field.

Cultural and Historical Perspectives: Some literature explores the cultural significance of rodents in various societies, as well as their historical roles in human agriculture and disease transmission.

To conduct a comprehensive literature review on rodents, it's essential to specify your focus within these broad themes and explore the most recent research, as the field continuously evolves.

ICMR NARFBR


भारतीय आयुर्विज्ञान अनुसंधान परिषद
INDIAN COUNCIL OF MEDICAL RESEARCH
NEW DELHI
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C R - R R

ntroduc on :

About IC R NAR BR Indian council of edical Researc Na onal Animal Resource acility for biomedical Researc was e lained by director

Sir Dr. raveen kumar Atul.

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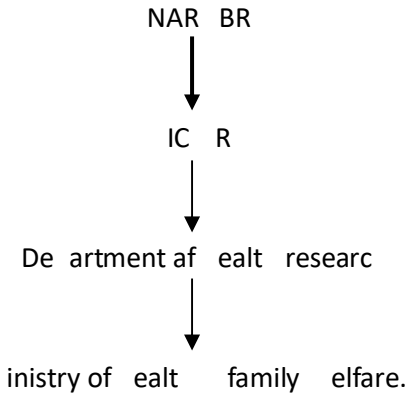

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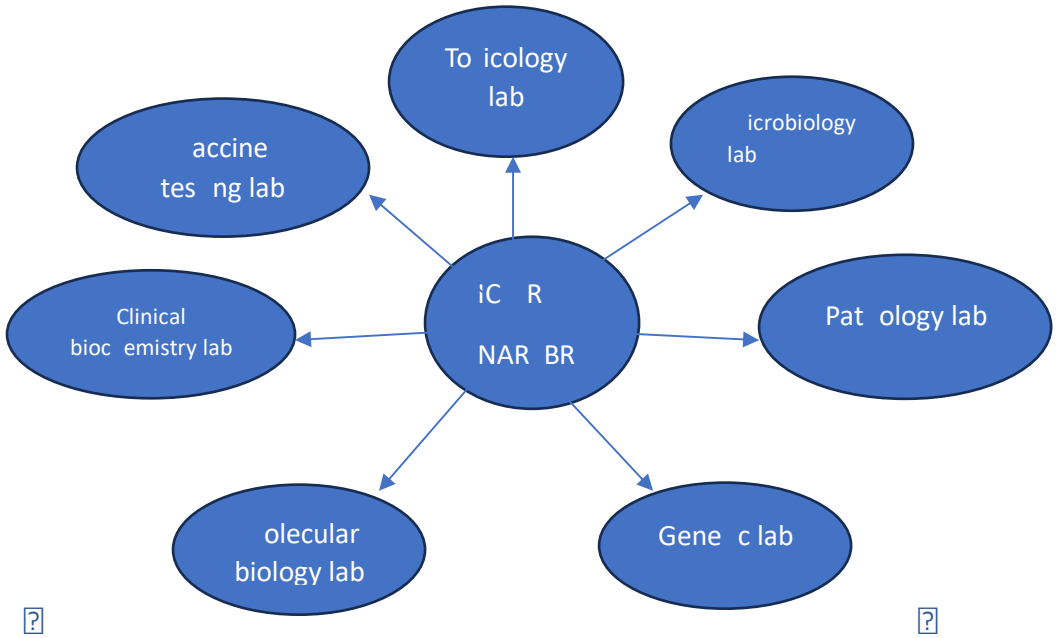
- ision far basic a lied regulatory researc in country

acili es :

Primate breeding facility
Primate e eriment facility
Primate indoor facility
Primate inde endent facility
Primate containment facility
Primate re abilita on facility
Equine quaran ne facility
Equine clinic facility
Equine e erimenta on facility
Equine bom facility
Small ruminants facility
Porcine facility

Biosafety level 3
Biosafety level



Rodents are Rats ice Guinea igs Rabbits.
Dogs are comes under canines facility
onkeys are comes under rimates facility
orses donkeys are comes under equines facility
Goats s ee s comes under small ruminants
Pigs comes under orcine

NOTE: Above men oned animals are resent in t e **C R R R**

Scope of C R:

- Researc
- Breeding
- uman resource develo ment
- Industrial interface.

Biomedical researc collaborates wit G countries

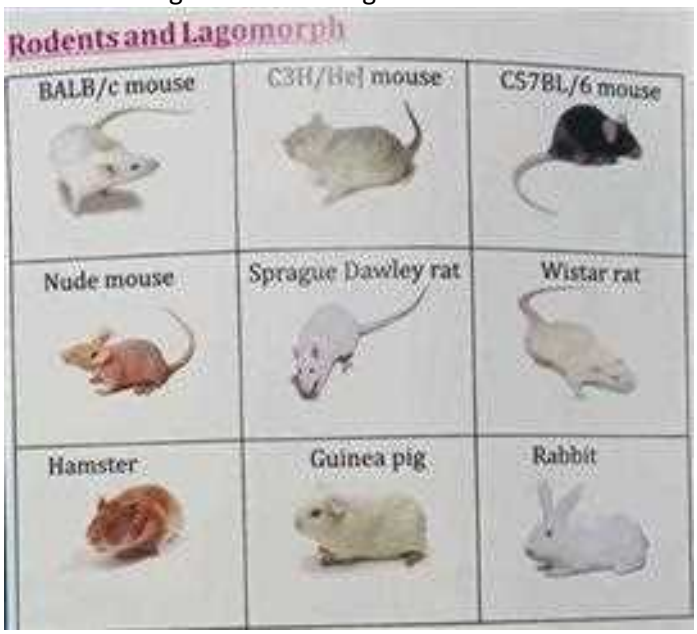
Conclusion:

T e nal conclusion for t is animal used for t e

- Preclinical tes ng researc develo ment
- edical device develo ment tes ng
- accine develo ment tes ng
- aintaining in laboratory animal science
- Drug tes ng develo ment etc

Rodent reeding acility:

Rodent Breeding acility is dedicated for breeding of SP quality rodents suc as rats mice amsters and Guinea igs as well as a lagomor t e rabbit.



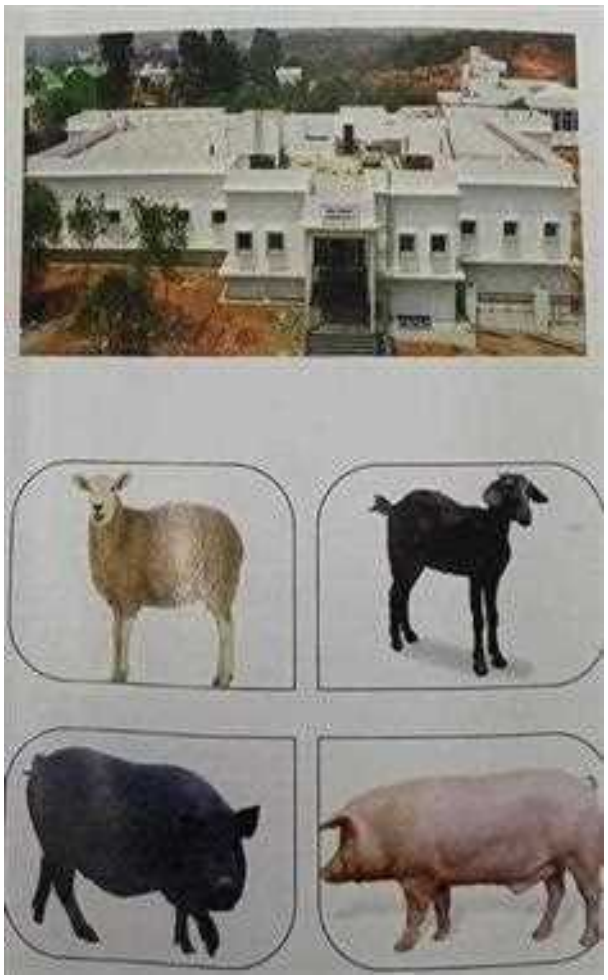
Rodent xperimenta on acility:

Dedicated for various ty es of e erimenta on suc as e loratory researc transla onal researc and re clinical tes ng on rats mice rabbits amsters and Guinea igs wit an advanced BSL facility.

Small Ruminants:

Small Ruminants and Porcine facility as goat and igs. Animals will be used for tes ng biomedical device an body roduc on cardio vascular re roduc ve and transgenic studies as well as model animals to study congenital diseases of umans.

T is facility also as dedicated surgical suite and ost o era ve room.



Canine breeding facility:

Canine facility at IC R NA BER as dedicated breeding and e eriment facility wit dedicated e ercise and lay area breeding facility as dedicated e ercise / lay area . breeding facility as t e ca acity to ouse beagle dogs

eagles dogs

Beagles are medium si ed and ave a s ort coat and an even tem erament w ic makes t em suitable model for biomedical researc

.

Canine experimenta on facility

Dedicated to conduct e erimenta ons suc as regulatory re clinical e lies biomedical devis Ses ng. armacological studies and be avioral studies

on-Human rimate acility

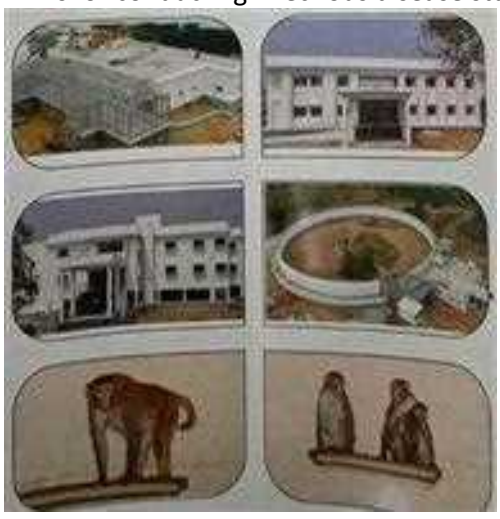
Non uman rimate N P facility as ve dedicated buildings vi indoor outdoor facilty. Individual ouses e erimenta on facility containment facility and re abilita on facility coral .

rimate reeding acility as t e ca acity to ouse N Ps wit required ameni es.

rimate xperimenta on acility as ca acity to ouse N Ps wit dedicated re and osto era ve care units.

Rehabilita on acility mainly for re abilita on of N Ps.

Containment acility wit a ca acity to ouse N Ps for conduc ng infec ous disease studies.



uine acility:

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T e Equine uaran ne acility of t e ins tute is dedicated for quaran ne of equines.

T e Equine Clinical Com le is dedicated for ealt care of equines.

Use of Equines in Biomedical Researc

orse as used as e erimental model for

y er immune sera genera on

Osteoart ri s

Tendino at y

Car lage In uries

Ast ma studies

Day: 2

Date: 19/07 2043.

Title: Animal Welfare, fore about Mouses and Visted housing system

1)clean coridor
2) Service coridor

Introduc on:

Animal Welfare

Animal: Living form of other than human right from Non- human primates to lower four's move and eat.

Welfare: General health, happiness of a person, an animal (or) a group.

Body: (Animal Welfare).

- Ensuring that all animals used by humans have their basic needs -ful lled in terms of food, shelter and health. They have experience in Providing for human heeds.
- The needs are proper housing, Managment, nutri on, disease preven on and treatment, responsible care

They are some

- Phyllosophy
- Principle
- Obliga on
- Applica ons

Phyllosophy :

- Discipline Comprising logic, ethics, aesthi cs (deputy of Work), meta physics (knowledge) and epistesmology
- A set of ideas or beliefs rela ng to a par cular eld or ac vity, an underlying theory

phyllocophy of Animal Welfare:

it endores the responsible use of animals to Sa sfy Certain human needs .

Phyllocophy th century

believed in transmigrator of 'soul through animal lives.

Basic phyllosophy in India thcentury :

Based on 'Ahimsa' propagated by believe of mainly ianism, buddhism and Hinduism

Aristotle BC 22 BC : lecutres on zoology in his academy called lyceaum & knowledge development

Emperior Asho a BC 2 2 5 i- prevented of killing animals and establish ver nary hospitals.

plutarch Epee A I2O) : oured Vegetarinism , did not support that animals to be prayed upon by man

Sainthonce. A uinas 22 2 AD : Vieuns of Aristotle

Nichael demon taige 5 99 - denounced any form Of Cruelty , towards humans /animals.

Kant German 2 : - denounced cruelty.

Kuth amison I9 : i-Avoidance of produc on of food animals.

Animal rights: (social jus ce issuse)

- Animals are not occurs to use for food, clothing, entertainment (or) experimenta on.
- Animal Welfare (ethical issues).

There uses as long as human gesture

Prinicipals of animal welfare

Five freedoms:

from thirst hanger and mal nutri on

From dis comfort due to environment.

- From ask ay pat, injury and disease.

From express normal behaviour for the species.

- From fear and dosstress.

Five welfare needs:

environment, Heath (diet) , diet, Behaviour, Camparion

Obliga ons :

Ar cle : protec on of cows, Ca le, mitch and drought animals.

5 Ar cle :protect and improve nature environment induced forest, lakes, rivers, wildlifeor for Iving Creatures.

Ar cle 9- Caddle fodden, including oil cakes & other.

Act & rules:

performing animal rules

transport of animal rules.

Applica ons in General:

Caring Al tude

Suppor ve approach

Preven on of facility

Veterinary Care & managerent

Conclusion:

Animal Welfare Was philosophical belief, moral colliga on and prac se since epical periods.

In comparison to animals

We are human being

Can We assume humanity

In absence of animal being

RODENTS

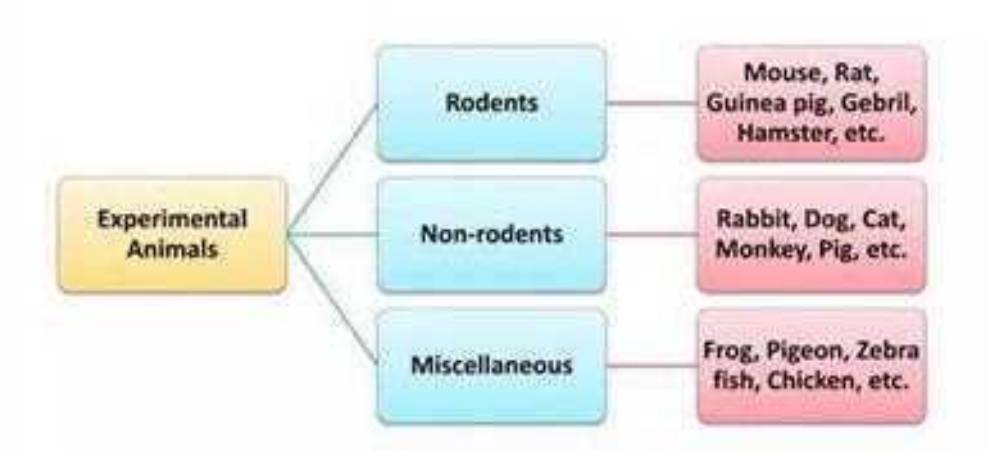
1. Introduction

Physiologically and anatomically there is similarity between the humans and animals at organs and organ systems, which functions in the similar fashion.

This similarity makes animal ideal for the study and development of products and techniques for humans.

By using laboratory animals, various discoveries have been made such as, diptheria and polio vaccine, insulin for the treatment of diabetes mellitus, heart valve replacement, antibiotic therapy, manic depressive drugs etc.

2. Classification of Experimental Animals



Most Commonly Used Laboratory Animals are:

- Rabbit
- Guinea pig
- Rat
- Mice
- Hamster

Different strains of Rabbit:

- Newzealand White

3. Description, handling and application of different species and strains of animals

RABBIT

Description-

- Rectal temperature:- 38.7°C -39.1°C
- Normal respiratory rate:- 55 per min
- Pulse rate- 135 per min
- Gestation period-28-31 days
- Weaning age- 6-8 weeks
- Mating age-6-9 months
- Litters:- 4 yearly, average 4 litter
- Room temp: 155-18.5
- Humidity 40-45 percent
- Weight-adult-0.9-6.75 kg

Housing:

- Cages are best made of galvanized iron.
- The minimum size for a medium sized rabbit is 2×2×1(1/2)ft.
- Young rabbits up to 3 months of age may be housed together but after that time sex should be separated.
- From 8-10 young rabbits may be kept together in pen similar to that used for guinea pigs.

Feeding:

- Pelleted diet 18 of Bruce and Parkers(1947) or commercial breeders pellets are suitable
- Daily supply of 72 gm of a mixture of one part oats and three parts bran may be fed as a slightly moist mash
- Green stuffs or root vegetables
- Clean drinking water

Handling:

- Smooth ear of the rabbit back
- pick up the ears and loose skin at the back of the neck with one hand in a firm grip
- place the other hand under the hind quarter to support the weight and lift gently.

- Never be lifted by ear alone
- Should be placed on a non-slippery surface ✓If restraint is required during anaesthesia or inoculation, should be wrapped in a roller towel or placed in a special box.

Common Diseases of Rabbits:

- Coccidioisis (hepatic and intestinal)
- Pseudo tuberculosis
- Respiratory infections(Snuffles)
- Pneumococci
- Streptococci

Experimental Procedures on Rabbits:

- Antisera
- Anaesthesia
- Scarification
- Subcutaneous inoculation
- Intra-venous inoculation

Applications:

- Pyrogen testing
- Bioassay of anti-diabetic, curare form drugs and sex hormones Screening of agents affecting capillary permeability .
- Drugs used in glaucoma
- Pharmacokinetic studies

3. Description, handling and application of different species and strains of animals

GUINEA PIG

Description-

- Rectal temp-37.6-389
- Normal respiration rate- 80 per minute
- Pulse rate 150 per minute
- Gestation period-59-72 days
- Weaning age: 14-21 days
- Mating age-12-30 weeks
- Litters: 3 yearly average litter3
- Room temp- 18.5-21
- Humidity-45
- Weight-weaning : 120g, adult 200-1000g

Housing:

- Stock runs should be abt. 4×6 ft and 1 ft. 8 in high
- One square foot of space should be allowed for each animal
- not more than 25 animals should not be kept in any one pen.
- For expt Animals galvanized iron cages are recommended and sterilized
- A convenient size of 14×9×8 in fitting in a tray 1.5 in deep

Feeding:

- A diet in pelleted form is recommended in preference to mashes.
- Diet of Bruce and Parks (1947) contains balanced proportions of protein, fats and carbohydrate with added vitamins salt and trace element.
- Crushed oats 2 part+ Broken bran I part
- Supplemented with cabbage and hay
- Necessary to add fish or meat meal

Different strains of guinea pig:

- Dinken Hatley

Handling:

- These are very humble rodents and can be easily handled because of their docile nature.
- Place one hand across the back of the animal with thumb behind the shoulder and the other fingers well forward on the opposite side
- Lift the animal gently and support its weight with other hand placed. palm uppermost under the hind quarters.

Common Diseases of Guinea pig:

- Pseudo tuberculosis (acute or chronic)
- Abscesses in lymphatic glands
- Respiratory tract infections
- intestinal infections
- Protozoan disease(Coccidioisis. Toxoplasmosis)

Experimental Procedures on guinea pig:

- Anasthesia Pentobarbitone sodium 28mg/kg-body weight)
- Subcutaneous inoculation
- Intracutaneous inoculation
- Intraperitoneal mnoculation
- Collection of blood

Applications:

- Evaluation of bronchodilators
- Anaphylactic and immunological studies
- Study of histamine and antihistamines
- Bioassay of digitalis
- Evaluation of local anesthetics

3. Description, handling and application of different species and strains of animals

RAT

Description-

- Typical adult weight - 250 g
- Average life span - 2-3yrs
- Gestation period - 21-23 days
- Average litter size-8-10
- Estrous cycle 4-5 days
- Heart rate-300-500 beats/min
- Resp rate-65-180/min
- Tidal volume-15 ml
- Daily food intake-10-20 g

Housing:

- Many different designs of rat and no one pattern is the standard
- Aluminium box approx 6×12×6 in deep with tapering side to facilitate stacking
- The lids are made of steel sheet or of strong wire mesh and are designed so that hopper is built into them and accommodation provided to hold the drinking bottle
- The cages are light, durable and easily sterilized by dry or moist heat.

Feeding:

- Pelleted diets such as diet 86 of Howie 1952) or diet 41 of Bruce (1950) are satisfactory
- Fresh water in drinking bottles must be provided

Different strains of Rat:

- Wistar
- Sprague Dawley

Handling:

- **Way 1:** Lift rat out of the cage by grasping the base of the tail and place on the soft urface. (Hard smooth surface can make rat tens.)
- **Way 2:** Place your index and middle finger along the rats head and your thumb and ring finger under its forelegs Use your index and muddle finger to secure its head and the remaining fingers to support the body
- **Way 3:** Hold the compete body by grabbin the back by usin complete palm.

Experimental Procedures on Rat:

- Study of analgesics and anticonvulsants.
- Study of oestrus cycle, mating behavior and lactation.
- Gastric acid secretion
- Hepatotoxicity studies
- Study on mast cells

Applications:

- Resembles man in several organ function and nutrition
- Sensitive to most of drugs, makes very usefull experimental animals
- Donot have vomiting centre (cant study about emetics)

3. Description, handling and application of different species and strains of animals

MOUSE

Description-

- Normal temperature -37.4
- Pulse rate: 120
- Estrous cycle:- 4-5 days
- Gestation period:-19-21 days
- Weaning age:- 19-21 days
- Mating age-6-8 weeks
- Litters 8-12 yearly
- Room temp 20-21
- Humidity 50-60%
- Weight weaning 7g adult- 25-28

Housing:

- Many different designs of mouse and no one pattern is the standard
- Aluminium box approx. 6×12×6 in deep with tapering side to facilitate stacking The lids are made of steel sheet or of strong wire mesh and are designed
- so that hopper is built into them and accommodation provided to hold the
- drinking bottle.
- The cages are light, durable and easily sterilized by dry or moist heat.

Feeding:

- Pelleted diets such as diet 86 of Howie(1952) or diet 41 of Bruce (1950) are satisfactory
- Fresh water in drinking bottles must be provided.

Different strains of Mouse:

- C57BL/6
- BALB/C
- SWISS albino

Handling:

- **Way 1:** One can handle it with the help of blunt foreceps by grasping the skin behind the neck/body. This technique is often used to transfer the mice from one cage to another.
- **Way 2:** Grasp the base of the tail with one hand and with other hand grasp the loose skin behind its neck. Way 3: Hold the compete body by grabbin the back by using all fingers.

Common Diseases of Mouse:

- Salmonellosis
- Ectromelia (mouse pox)
- Streptobacillus moniliformis infection
- Miscellaneous virus infection
- Worms(Taenia taenia- formis)

Experimental Procedures on Mouse:

- Anasthesia
  - short acting : Ether
  - Long acting : Pentobarbitone sodium
- Subcutaneous inoculation
- Intraperitoneal inoculation
- Intracerebral inoculation
- Intravenous inoculation

Applications:

- Toxicological studies
- Teratogenicity studies
- Bioassay of insulin
- Screening of analgesic and anticonvulsant
- Screening of chemotherapeutic agents

Laboratory Rodents

Laboratory rodents:

1) Rat (Rattus norvegicus)

2) Mouse (Mus musculus)

3) Guinepig (porcellus)

4) Rabbit (oryctolagus)

Reproduction &Breeding :

1) Age of animal

2) Health status

3) Genetic pubert af Animal.

Introduction:

laboratory rodents.

It is largest mammalian group approximate 1500 species nearly 401 of all mammalian species are rodents.

• It has single pair of upper & lower incissors Present

eg:- pygmy mouse.

BODY:

It belongs to the Muridae family such as Rats, mice, hamsters 1138 of its phylogenetic tree,

Rodents as lab animals:

Mouse, rats, guinea pig hamster, dusmice , chinchillas and Cotton rats, gerbils.

They are transgenic (introduce foreign gene into animal).

micro injection method:-

Introduction of gene into bacteria called translation Introduce gene into plant/animal called transection.

- urithin group all animals same called Inbred.
- With in group all animals different Called outbred

❖ Reproduction and Breeding.

Introduction: 3 categories

1)Age of animal

2) Health status

3) Genetic puberty of animal.

Body:

Some information about the gestation period and genetically modified species.

In mouse:

Ho chromosomes

H2 days of puberty

19-21 days gestation period

4 days of oestrous cycle.

In Rat

42 Chromosomes

50-60 days of puberity

21-23 days gestation period

4 days of Destrous cycle

Guinea pig (caviaporcellus)

64 chromosomes

68-72 days gestation period

15 days estrous cycle

60-70 days of puberty

In rabbit

44 chromosomes

2.5 kgs in adults

30 days of gestation period

No estrous cycle

6 months of pubertiy

Estrus cycle:

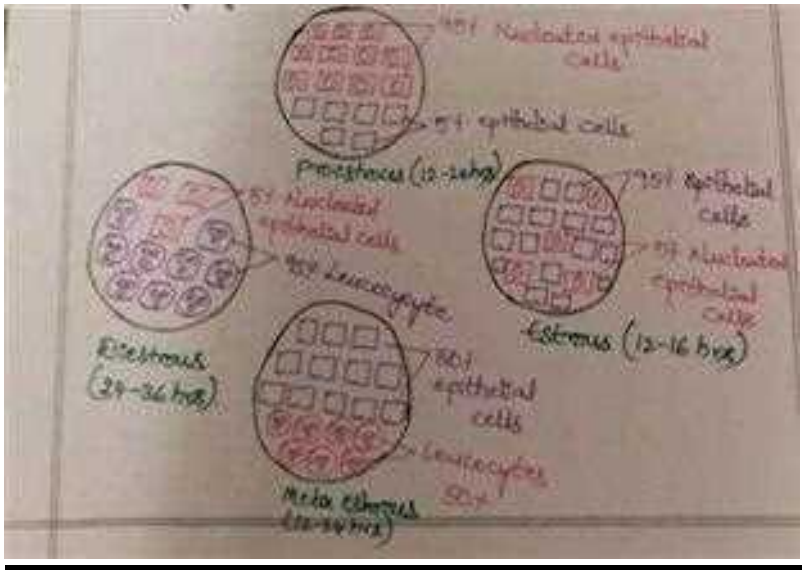
1)proestrous

2)estrous

3)metaestrus

4)distrous

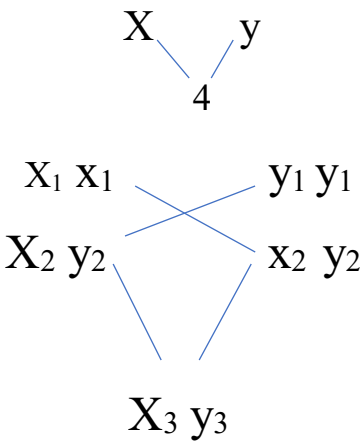
Identity by smear :



Conclusion :

Poresding mating method:

1) monogamous: male and female



2)**polygamous**: 2 female (or) more with 1 female

3)**haram mating**: eg use for guinea pigs because they are communal animal

1 male – 6 famales in truff

Cannabolism: eating own pubs

4) **hand mating method /color method:**

Taking female to male

Eg:rabbit & hamster (male 5 -10min)

Rabbits super ovulation/spomtaneous ovualtion

LABORATORY RODENTS AND RABBITS E PERIMENTAL PROCEDURE AND WELFARE.

Introduc on.

- **Animal model.**
  It is use for scien c purpose.
  It is a living Organism in norma ve biology, Spontaneous (or) induced pathological process.

History:

Louis Pasteur used dog brain for func on Aristotle used chick embryo called embroyogenesis William Harvey studied cardio vascular used deer /stalk.

Emilvon used some animals, guinea pigs for diphtheria disease

Animal models :

- Ring discovery
- Drug tes ng
- Pathogenesis of disease
- accine development
- educa on straining
- Toxicological research
-

Procurement and uaran ne of animals

Ins tu on of Animal ethics commi ee (AEE) approval is mandatory

uaran ne period: At least 1 Week.
Directorate General of Foreign trade (DGFT). Commi ee for purpose of Contral supervision of experiments on Animals (cpcsen).

Handling Of laboradory mice.

Approach animal Calm and Con dent

1) **Handling tunnels** (pipe). Approach animal calm put into cage transfer animal to other Cage.
2) Cupping: Scoop the mouse onto the hand for smaller
3) Rats: Turner, Elapsing around shoulder.
) Rabbits: scru method (below the ears).

trun area: - Grap the animal With towel, Collect blood by artery

Guinea pigs -shoulder method, support hind limb

Iden ica on of laboratory animals: staining, lagging, ta ooing, notching

Routes of substance adminstra on in lab animals

- Intra muscular in ect
- Intra muscular enous.
- Intra dermal
- Intra peritonia
- Intra subcutaneous
- Retro Orbital
- Intra tracheal
- Intranasal

by mouth.

Blood collec on in lab animals.

depends upon the body size

In mouse: Retro Orbital

In Rat: Tail, Retro orbital

Rabbit: earvein Cardiac

Anesthesia in lab animals (unconsions).

1) In ectable Anesthesia.

Drug given by intravenously

chitamin and zyrasin- Using sede on of animal.

It is for muscle relaxa on.

2) In hern Anesthesia

use Iso uoride

Eultanesia (sacri ce).

during death without pain

1) **physical method & cerical method** (for mouse and rat)

2)**inhala on of grass** co_2,co

3) **drug**

Conclusion: -

For posi ve regula on of hepa s irus replica on by micro RNA- 122 this virus e ects mainly the triester month of pregnant woman.

For experimental procedures Welfare is for Animal rights is equal nights with human.

Not use for their by products.

Animal Welfare Ethical

Human treatment for amimals.

Three R S:

1)replacement

2)Re nement (minimize pain)

3)reduc on (reduce no of popula on).

CANINES BREEDING FACILITY

Canines, also called canids, include foxes, wolves, jackals, and other members of the dog family (Canidae). They are found throughout the world and tend to be slender long-legged animals with long muzzles, bushy tails, and erect pointed ears. This is a list of canines ordered alphabetically by genus.

DOG (Canis lupus familiaris)

Kingdom: Animalia

Phylum: Chordata

Class: Mammalia

Order: Carnivora

Family: Canidae

Genus Canis

Species: C. lupus familiaris

Breed that are used in icmr-narfbr: Beagle

● Beagles are medium-sized and have a short coat and an even temperament, which

makes them particularly suitable for medical research in contrast to other breeds.

● Beagle dogs belongs to Hound breed group

● Since they are physiologically more similar to humans than other species used in

research and they share similar environmental conditions.

Physical characteristics:

i. Birth weight: 140- 280 g

ii. Adult Height: 13- 15 inches

iii. Adult Bodyweight:10–12 kg

(males), 9–10 kg (females)

iv. Coat length: Short

Life history & gestation parameters:

i. Life span: 10- 15 years in captivity

ii. Weaning age: 45- 65 days

iii. Sexual and social maturity: 10 months

iv. Gestation: 60 and 65 days

v. Litter size: 5-6 pups

Food for Beagles:

- Food consumption: 25-40 gm/kg/day; divided into two meals per day.

-Commercially available pellet feed – Royal canine & Pedigree.

-Mainly involved in basic, educational, regulatory, and contract researches.

● Used to test the safety of drugs, medical devices and pesticides.

Veterinary care

● By dedicated veterinary and staff members- as per the regulatory and welfare

standards.

● Ensures macaques are provided nutritious diet, and behavioral enrichment activities.

● Monitoring animal care and welfare.

● Maintenance of clean, safe environments.

● Prevention, diagnosis, treatment, and control of disease.

Why are beagle dogs used for research?

The most common breed of dog used for experiments are beagles, but not because scientists view them as the best model for human disease. Rather, beagles are convenient to use because they are docile and small, allowing for more animals to be housed and cared for using less space and money.

During the Humane Society's investigation, beagles underwent tests for **cancer drugs** that reportedly caused diabetes, as well as tests for **painkillers**, a drug for **Hepatitis B** and a medication for **Cushing's disease**

Speciality of beagle dog: Beagles are excellent dogs for hunting rabbits and hares. They have a phenomenal sense of smell and seemingly endless stamina.

BSL 3 Safety Protocols

BSL 3 stands for Biosafety Level 3, which is a containment facility designed to safely handle and study infectious agents that can cause serious or potentially lethal diseases in humans. These labs have strict safety protocols to prevent the release of dangerous pathogens into the environment and protect the researchers working with them. Is there anything specific you would like to know about BSL 3

❖ Applications of bsl3

BSL 3 labs have various important applications, including:

Research on Highly Infectious Diseases: BSL 3 facilities are essential for studying and understanding pathogens like tuberculosis, anthrax, SARS-CoV-2 (the virus responsible for COVID-19), and other potentially lethal agents. Scientists use these labs to conduct experiments to better understand the diseases and develop treatments or vaccines.

Developing Vaccines and Therapeutics: BSL 3 labs play a crucial role in the development of vaccines and therapeutic agents against infectious diseases. Researchers can test potential vaccines and treatments on live pathogens under controlled conditions to evaluate their effectiveness and safety.

Diagnostics and Surveillance: BSL 3 facilities help in diagnosing and identifying infectious diseases accurately. Scientists can work with live samples while following strict safety measures, aiding in disease surveillance and outbreak investigations.

Public Health Preparedness: These labs are essential for enhancing public health preparedness by studying and preparing for potential pandemic threats. By researching high-consequence pathogens, experts can develop strategies to respond effectively to outbreaks.

Training and Education: BSL 3 labs provide a controlled environment for training researchers, healthcare professionals, and first responders on how to work safely with dangerous pathogens, ensuring they understand the proper procedures and protocols.

Veterinary Research: BSL 3 facilities are also used in veterinary research to study infectious diseases that can affect animals and, in some cases, transmit to humans. Understanding zoonotic diseases is crucial for preventing outbreaks and protecting both animal and human health

These applications demonstrate the importance of BSL 3 labs in advancing scientific knowledge, public health, and disease management.

Types of bsl3

Biosafety Level 3 (BSL 3) facilities can be categorized into different types based on their specific features and capabilities. Here are the main types of BSL 3 labs:

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Research BSL 3: These labs are primarily dedicated to conducting scientific research on infectious agents. They focus on studying the biology, transmission, and host-pathogen interactions of high-consequence pathogens to develop treatments and vaccines.

Animal BSL 3: These facilities are designed to study infectious diseases that affect animals and can be transmitted to humans. Veterinary researchers use them to investigate zoonotic diseases and assess their potential impact on public health.

Maximum Containment BSL 3 (BSL 3-Ag or BSL 3-Ag/Animal): These are the highest-level BSL 3 labs, and they are equipped to handle large

animals infected with highly dangerous pathogens. These labs are used for research involving agricultural and zoonotic agents.

Regional/Public Health BSL 3: These BSL 3 facilities are part of public health infrastructure and are intended to support regional responses to infectious disease outbreaks. They contribute to surveillance, diagnosis, and research efforts during public health emergencies.

Each type of BSL 3 facility serves specific purposes, from medical treatment and research to safeguarding public health and enhancing our understanding of infectious diseases. The level of containment and safety measures increases accordingly, ensuring the protection of both laboratory workers and the broader community.

Materials of bsl3

Biosafety Level 3 (BSL 3) laboratories require specialized materials and equipment to provide a safe working environment when handling highly infectious agents. Some of the essential materials and features commonly found in BSL 3 facilities include:

Personal Protective Equipment (PPE): This includes full-body suits, gloves, respirators, face shields or goggles, and shoe covers to protect laboratory workers from direct contact with pathogens.

Laboratory Furniture and Fixtures: The lab is equipped with smooth, non-porous surfaces that are easy to clean and disinfect. Special attention is given to the design of workbenches, biosafety cabinets, and waste disposal units.

Air Handling Systems: BSL 3 labs have advanced ventilation systems to maintain negative air pressure and ensure the airflow directs away from the outside environment. These systems help prevent the release of infectious agents into the surrounding areas.

Biosafety Cabinets (BSC): BSCs are essential for handling infectious materials inside the lab. They provide a contained and ventilated workspace for tasks such as sample preparation and culturing.

Autoclaves: These are used for sterilizing laboratory equipment and waste before disposal to ensure that no viable pathogens are released.

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Decontamination Procedures: Rigorous decontamination procedures are employed to ensure that the lab and all equipment are free from any residual pathogens after use.

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BSL-3 Advantages & Disadvantages

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Biosafety Level 3 (BSL-3) facilities offer several advantages, including:

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Reduced risk of exposure: By using strict protocols and safety measures, BSL-3 facilities minimize the risk of accidental exposure to dangerous pathogens.

Research flexibility: BSL-3 labs enable researchers to study a wide range of infectious agents and conduct experiments that require higher containment.

Preventing outbreaks: These facilities play a critical role in identifying and studying potential outbreaks, helping to develop treatments and preventive measures.

Training opportunities: BSL-3 facilities offer valuable training opportunities for researchers, preparing them to work with hazardous agents safely.

Compliance with regulations: Working in a BSL-3 lab ensures compliance with biosafety and biosecurity regulations set by authorities.

It's important to note that while BSL-3 facilities have numerous advantages, they also require strict adherence to protocols and safety guidelines to prevent accidents and ensure the well-being of laboratory personnel and the community.

❖ Disadvantage of bsl3

While Biosafety Level 3 (BSL-3) facilities offer important advantages, they also come with several notable disadvantages:

Costly and complex infrastructure: Setting up and maintaining a BSL-3 laboratory requires significant financial investment due to the need

for specialized equipment, stringent safety features, and ongoing maintenance.

High operational expenses: Operating a BSL-3 facility can be expensive due to the extensive safety measures, rigorous training, and ongoing monitoring required to ensure the safety of personnel and prevent pathogen release.

Limited accessibility: BSL-3 labs are not as widely available as lower-level labs, limiting the accessibility of specialized research and testing capabilities for many institutions and researchers.

Training requirements: Working in a BSL-3 environment demands extensive training for personnel, which can be time-consuming and may limit the number of qualified researchers.

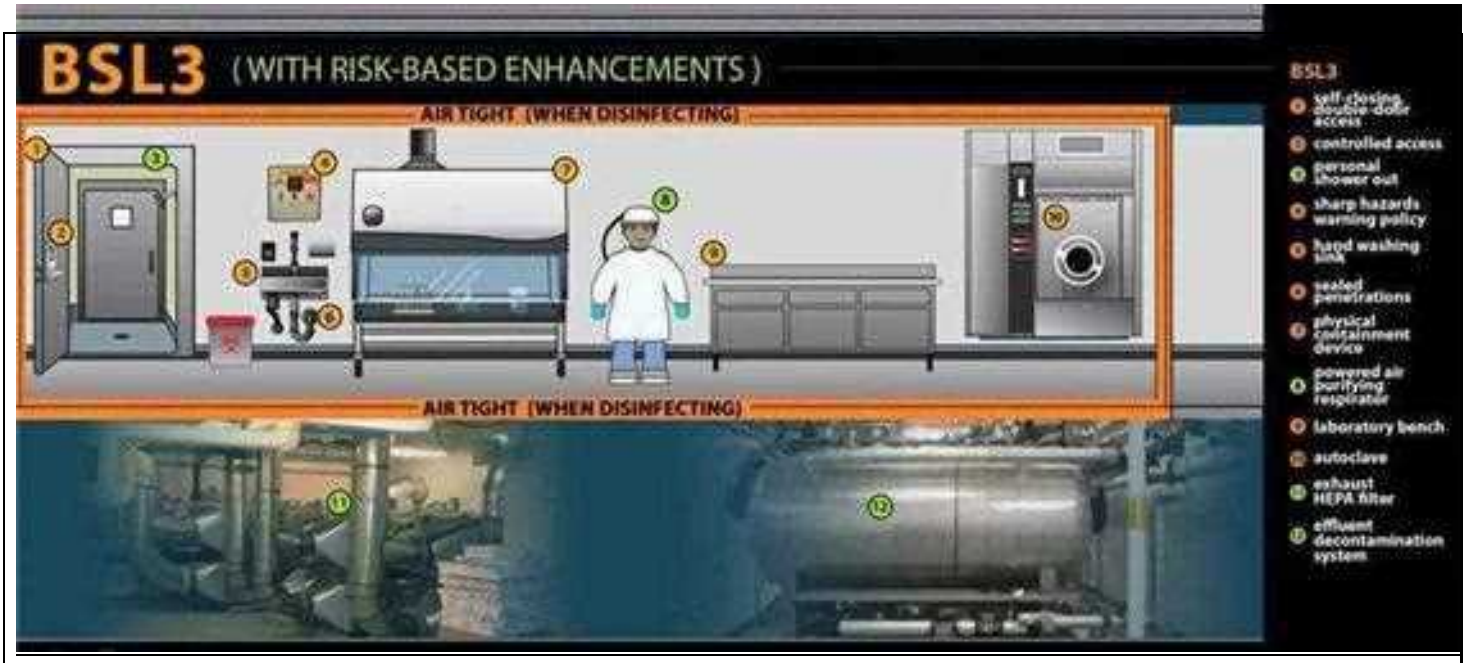
Reduced workflow efficiency: The use of protective gear and adherence to strict safety protocols can slow down research processes and experiments, leading to reduced workflow efficiency.

Potential for cross-contamination: Despite rigorous safety measures, there is always a risk of accidental pathogen release or cross-contamination, which could have severe consequences if not properly managed.

Psychological stress: Working in a high-containment environment can be mentally demanding for researchers due to the constant awareness of potential risks and the need for strict adherence to safety protocols.

Public perception: BSL-3 labs can be a source of concern for nearby communities due to fears of potential pathogen release or accidental exposures, which may lead to public opposition or regulatory hurdles.

These disadvantages highlight the importance of carefully considering the necessity of BSL-3 facilities and implementing robust safety measures to minimize risks and ensure the safe handling of hazardous agents.



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In conclusion, Biosafety Level 3 (BSL 3) facilities play a critical role in safeguarding public health by providing a high level of containment and safety when handling highly infectious agents. These labs are equipped with specialized materials, equipment, and protocols to protect researchers, prevent the release of dangerous pathogens, and advance scientific knowledge. BSL 3 labs are used for various purposes, including research on infectious diseases, vaccine development, diagnostics, and preparedness for potential outbreaks.

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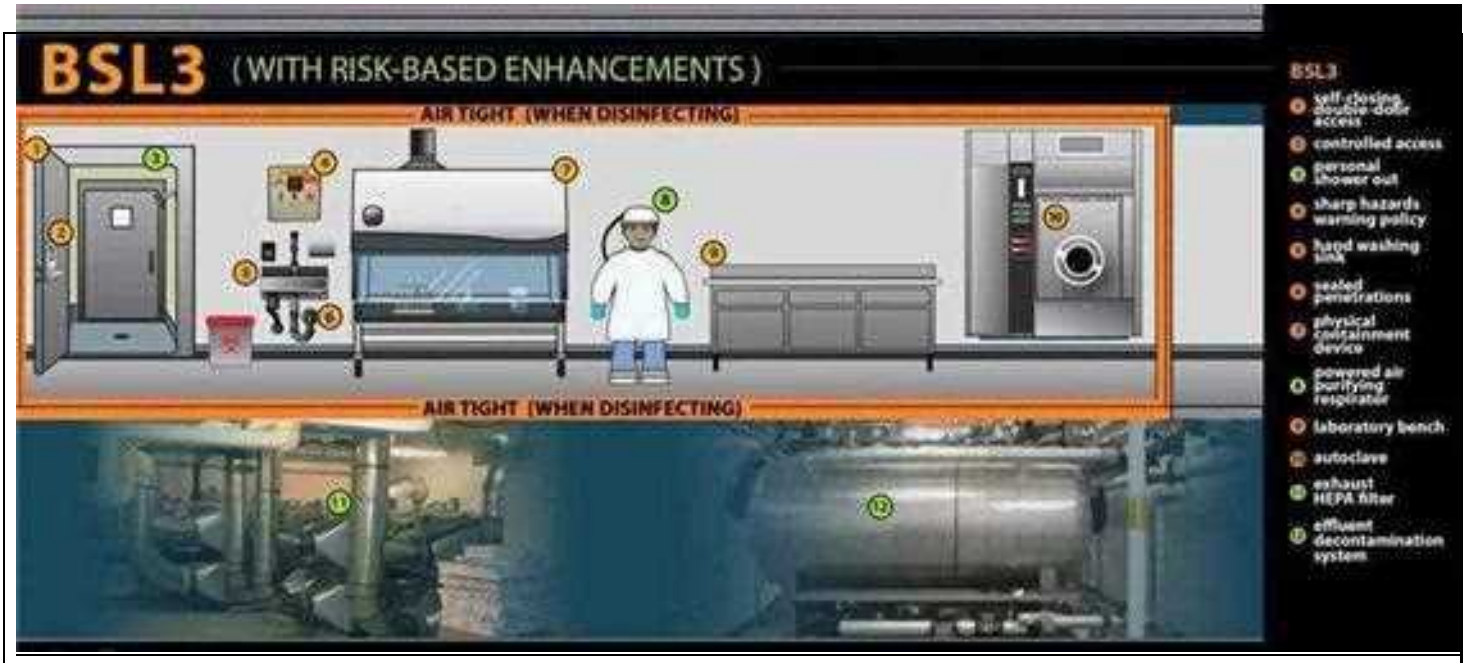

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CONCLUSION

In conclusion, which I have learned from ICMR NARFBR institute is very valuable to me. I have learnt how animals are used in biomedical research. Also leamt many valuable information about the laboratory animals such as animal handling techniques, feeding stuff of animals, breeding techniques and experimentation techniques. I also learnt some techniques such as PCR, Agarose gel electrophoresis, Indirect ELISA, DNA extraction and Isolation, Blood collection from mice, organ collection from Rat. Also New drug research and tests to ensure the quality and efficacy of pharmaceutical products/ vaccines / biological research require animal experiments. The institute proposes to breed specific pathogen free large and small animals such as mice, rats, hamsters, rabbit, guinea pigs, canines, equines, sheep, and goats. Various species of non-human primates such as rhesus monkey among others needed for research purpose.

Reference

Certainly, if you re looking for a comprehensive reference on rodents, you might nd these books and scien c sources helpful:

Rodents of the World by Richard W. Thorington r. and ohn L. oprowski - This book provides detailed informa on about the diversity, biology, and distribu on of rodents worldwide.

Walker s Mammals of the World by Ronald M. Nowak - While not solely about rodents, this renowned reference book covers all mammal species, including rodents, with extensive informa on on their taxonomy, behavior, and distribu on.

The Behavior of Rodents: Mechanisms and Func ons edited by Per Andersen - This scien c book delves into the behavior and ecological roles of rodents, o ering insights from various researchers in the eld.

ournals and ar cles - Scien c journals like the ournal of Mammalogy, Mammal Review, and the ournal of Rodentology publish research ar cles on rodents, providing up-to-date informa on on their biology and ecology.

nline databases - Websites like the Smithsonian Na onal Museum of Natural History s Roden a database or the IUCN Red List of Threatened Species are valuable online resources for informa on on speci c rodent species.

A Study on Tilipia Fish Excreting Material As A Source Nutrients For Aquaponics Systems

A project report is submitted To

Department of Biosciences and Biotechnology

M.SC.Zoology

By

K. Taraka Rama Naik

Project Advisor

Dr. Ch. Suresh Babu

&

Dr. J.NaveenaLavanyaLatha

University college of Arts and Sciences

Krishna University

Rudravaram- 521001, Machilipatnam, Andhra Pradesh. India

DECLARATION

I HERE DELARE THAT RESEARCH WORK EMBODIED IN THIS PROJECT REPORT ENTITLED A STUDY ON TILAPIA FISH EXCRETING MATERIAL AS A SOURCE NUTRIENTS FOR AQUAPONICS SYSTEMS AT MACHILIPATNAM ANDHRA PRADESH INDIA. SUBMITTED TO KRISHNA UNIVERSITY FOR PROJECT WORK IN ZOOLOGY , IS THE OUT COME OF INVESTIGATION CARRIED OUT BY ME AS PROJECT WORK UNDER THE SUPERVISION OF **DR.CH.SURESH BABU & DR. J.NAVEENA LAVANYA LATHA** DEPARTMENT OF BIOSCIENCES AND BIOTECHNOLOGY, KRISHNA UNIVERSITY RUDRAVARAM, MACHILIPATNAM. I ALSO AFFIRM THAT THE PROJECT WORK IS ORIGINAL AND HAS NOT BEEN SUBMITTED TO ANY OTHER UNIVERSITY OR INSTITUION.

PLACE : MACHILIPATNAM

DATE :

NAME: K.TARAKA RAMA NAIK

CERTIFICATE

This is to certify that the research work described in this project report entitled "A Study On Tilapia Fish Excreting Material As A Source Nutrients For Aquaponics Systems **at Machilipatnam , Andhra Pradesh India** *is the outcome of work carried out by* **Mr.Taraka Rama Naik Kelavathu Msc Zoology Roll no:** *Y21ZOO101010 project student, University college of Arts and Science,Krishna University ,Rudravaram , Machilipatnam , Andhra Pradesh , India.*

Place : Machilipatnam

Date :

20/3/2023

Dr. J.N.LavanyaLatha

(Project advisor)

Dr. Ch Suresh Babu

(Project advisor)

Head of the Department

HEAD
DEPARTMENT OF BIOSCIENCES & BIOTECHNOLOGY
UNIVERSITY COLLEGE OF ARTS & SCIENCE
KRISHNA UNIVERSITY
MACHILIPATNAM - 521 003. A.P

ACKNOWLEDGMENTS

This project report would not have been possible without the expert guidance of my esteemed advisor **Dr. Th. Suresh Babu** *Department of Zoology , krishna University. Her responded to the drafts of each chapter of my work more quickly than I could have hoped for taking timeout from her busy schedule. Her written comments are always extremely perceptive, helpful and valuable suggestions throughout the tenure of the work. I am fortunate to have been blessed with privilege of working under her research supervision.*

I must also acknowledge to , **Dr. J. NaveenaLavanyaLatha** *faculty member of Department of Bio Technology, krishna university, Machilipatnam for motivating me into the pursuit of research by making learning interesting. Her becames the source of guidance, consultation and advice.*

I am extremely thankful to **Dr. I. SuseelaHOD** *and other faculty members of Department of Biosciences and Biotechnology, Krishna University, Machilipatnam for India for their guidance whenever necessary*

PREFACE

Recirculating aquaculture–hydroponic systems were designed to provide an artificial, controlled environment that optimizes the growth of fish (or other aquatic species) and soil-less plants, complete control of water quality, the production schedule and the fish product, while conserving water resources. Nutrients removal such as inorganic nitrogen and phosphate is essential for aquaculture wastewater treatment to protect receiving waters from eutrophication as well as for potential reuse of the treated water. In this study, a prototype of an aquaponic system was built at the Freshwater Hatchery Unit on the Krishna University Machilipatnam . The system consists of a fish culture tank, hydroponic trough, sump, sand filter and water holding tank. Hydroponic troughs were planted with water **spinach (Ipomoea aquatica**) **Oryzasativa(Rice plant) , Solanumlycopersicu (tomato) , Amaranthus**that been used to treat wastewater from an aquaculture system stocked with **Tilipia fish** . The unplanted hydroponic trough was concurrently run as a control unit. The effect of five different water flow rates was tested in order to relate nutrients removal, water quality with plant growth. The results showed that the aquaponic recirculating system removed 5-day biochemical oxygen demand (47–65%), total suspended solids (67–83%), total ammonia nitrogen (64–78%), and nitrite-nitrogen (68–89%), and demonstrated positive correlated with flow rates. Total phosphorus and nitrate-nitrogen removal rates varied from 43% to 53% and 42% to 65%, respectively, and were negatively correlated with flow rates. It was found that all flow rates were efficient in nutrient removal and in maintaining the water quality parameters within the acceptable and safe limits for growth and survival of fish.

Contents

1. INTRODUCTION

Aquaponics is a sustainable and integrated farming system that combines aquaculture (fish farming) and hydroponics (soilless plant cultivation). In aquaponics, the waste produced by aquatic organisms, such as fish, serves as a valuable source of nutrients for the growth of plants. This study focuses on one of the key components of aquaponic ecosystems: the utilization of tilapia fish excreting material as a nutrient source.

Tilapia (Oreochromis spp.) are one of the most commonly used fish species in aquaponics due to their hardiness, rapid growth, and adaptability to varying environmental conditions. In this research, we aim to explore the potential of tilapia excreting material, primarily composed of ammonia and other nitrogenous compounds, as a sustainable and cost-effective nutrient source for the associated hydroponic plant system.

The study will delve into the following key aspects:

Nutrient Cycling: Understanding the natural process of nutrient cycling within an aquaponics system, where fish waste is broken down by beneficial bacteria into forms that can be readily absorbed by plants.

Nutrient Content: Analyzing the composition of tilapia excreting material to determine its nutrient content, with a focus on nitrogen, phosphorus, and other essential elements required for plant growth.

Plant Growth Performance: Evaluating the impact of tilapia waste-derived nutrients on the growth and yield of various hydroponically cultivated crops, such as Oryzasativa (Rice plant) , Solanumlycopersicu (tomato) , Amaranthus

Sustainability and Cost-efficiency: Assessing the sustainability and economic feasibility of using tilapia excreting material as a primary nutrient source compared to traditional hydroponic nutrient solutions.

Environmental Benefits: Investigating the environmental benefits of reducing the reliance on synthetic fertilizers in hydroponics by utilizing fish waste, potentially lowering the ecological footprint of aquaponic systems.

In summary, this study aims to contribute to the knowledge base of aquaponics by exploring the potential of tilapia fish excreting material as a sustainable and nutrient-rich source for hydroponic plant cultivation. The findings could have implications for improving the efficiency and sustainability of aquaponic systems, ultimately benefiting both food production and environmental conservation efforts.

2. Literature Review

Effect of flow rate on water quality parameters and plant growth of Oryzasativa(Rice plant) , Solanumlycopersicu (tomato) , Amaranthus in an aquaponic recirculating system

Recirculating aquaculture–hydroponic systems were designed to provide an artificial, controlled environment that optimizes the growth of fish (or other aquatic species) and soil-less plants, complete control of water quality, the production schedule and the fish product, while conserving water resources. Nutrients removal such as inorganic nitrogen and phosphate is essential for aquaculture wastewater treatment to protect receiving waters from eutrophication as well as for potential reuse of the treated water. In this study, a prototype of an aquaponic system was built at the Freshwater Hatchery Unit on theKrishna University Machilipatnam . The system consists of a fish culture tank, hydroponic trough, sump, sand filter and water holding tank. Hydroponic troughs were planted with water **spinach (Ipomoea aquatica**) **Oryzasativa(Rice plant) , Solanum lycopersicu (tomato) , Amaranthus**that been used to treat wastewater from an aquaculture system stocked with **Tilipia fish**. The unplanted hydroponic trough was concurrently run as a control unit. The effect of five different water flow rates was tested in order to relate nutrients removal, water quality with plant growth. The results showed that the aquaponic recirculating system removed 5-day biochemical oxygen demand (47–65%), total suspended solids (67–83%), total ammonia nitrogen (64–78%), and nitrite-nitrogen (68–89%), and demonstrated positive correlated with flow rates. Total phosphorus and nitrate-nitrogen removal rates varied from 43% to 53% and 42% to 65%, respectively, and were negatively correlated with flow rates. It was found that all flow rates were efficient in nutrient removal and in maintaining the water quality parameters within the acceptable and safe limits for growth and survival of fish.

2.1 TILAPIA *:*

Background

Tilapia is native to Africa and Middle East, and has emerged from mere obscurity to one of most productive and internationally traded food fish in the world . The farming of tilapias especially of Nile tilapia (Oreochromisniloticus) in its curdest form is believed to have originated more than 4000 years ago from Egypt. The first recorded scientifically oriented culture of tilapia was conducted in Kenya in 1924 and soon spread to many parts of the world.

The uncontrolled breeding of tilapia in ponds, which led to excessive recruitment, stunting and a low percentage of marketable-sized fish, dampened the initial enthusiasm for tilapia as a food fish. However, the development of hormonal sex-reversal techniques in the 1970s, followed with research on nutrition and culture systems, along with market development and processing advances, led to rapid expansion of the industry since the mid-1980s. Though several species of tilapia are cultured commercially Nile tilapia is the predominant cultured species worldwide.

The last three decades have seen significant developments in farming of tilapias worldwide. They are being farmed in about 85 countries worldwide (FAO, 2008) and about 98% of tilapia produced in these countries are grown outside their original habitats (Shelton, 2002). Global tilapia aquaculture production in 2009 was 3.08 million mt, with China, Indonesia, Egypt and the Philippines being the top producers.

Species for aquaculture

Tilapia belongs to the family Cichlidae under order Perciformes. The tilapias have recently been classified into three genera, based on parental incubation of eggs. The species of the genera Sarotherodon and Oreochromis are mouth brooders, while Tilapia incubates eggs in a lake or pond bottom built-in "nest". There are about 70 species of tilapias, of which nine species are used in aquaculture worldwide (FAO 2008). Important commercial species include: the Mozambique tilapia (Oreochromismossambicus), blue tilapia (O. aureus), Nile tilapia (O. niloticus), Zanzibar tilapia (O hornorum), and the red belly tilapia (O. zilli).

Habitat and biology

Nile tilapia is a tropical species that prefers to live in shallow water. It is an omnivorous grazer that feeds on phytoplankton, periphyton, aquatic plants, small invertebrates, benthic fauna, detritus and bacterial films associated with detritus. Sexual maturity in ponds is reached at an age of 5-6 months. Spawning begins when the water temperature reaches 24°C. The female incubates the eggs in her mouth and broods the fry after hatching until the yolk sac is absorbed. Fecundity is proportional to the body weight of the female. A 100 g female will produce about 100 eggs per spawn, while a female weighing 600-1000 g can produce 1000 to 1500 eggs. Nile tilapia can live longer than 10 years and reach a weight exceeding 5 kg.

Farming systems

Tilipia farming ranges from a rural subsistence (extensive low input practices, non-commercial and for household consumption) to a large scale (capital intensive, commercial purpose and market driven) level, depending on the intensity of management employed

Intensive Tanks

Intensive tank culture avoids with over breeding because there is no space for males to set up territories. It requires a constant supply of water, either gravity fed or pumped. Usual maximum stocking rates in tanks where the water is changed every 1-2 hours would be around at 25-50 kg/m3

Potential for tilapia culture in India

As the demand for fish is increasing, diversification of species in aquaculture by including more species for increasing production levels has become necessary. Introduction of tilapia in our culture systems is advantageous because it represents lower level in food chain, and thus its culture will be economical and eco-friendly. Mono sex culture of tilapia is advantageous because of faster growth and larger and more uniform size of males.

The development of Genetically Improved Tilapia (GIFT) technology is based on traditional selective breeding and is meant to improve commercially important traits of tropical farmed fish which is a major milestone in the history of tilapia aquaculture. Through combined selection technology, the GIFT program achieved 12-17% average genetic gain per generation over five generations and cumulative increase in growth rate of 85% in O. niloticus (Eknath and Acosta, 1998). Other varieties like ‘red tilapia ‘ also hold promise. There is high potential of export tilapia to US, Europe and Japano

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Fig : 1 :- Tilapia fish

2.2 Ammonia:

The Role of Ammonia in Aquaponics Systems

Ammonia plays a significant role in an aquaponics system. It starts the nitrogen cycle and is an engine to your system's ecology. Fish produce waste that is full of ammonia. Bacteria convert them into nitrites and then nitrates necessary for plant growth. Fish produce ammonia to your aquaponics system through their faeces and from their gills. Understanding the role of

ammonia in your aquaponics system is very important, and hopefully, this post will help you understand the role of ammonia in your system.

What is Ammonia?

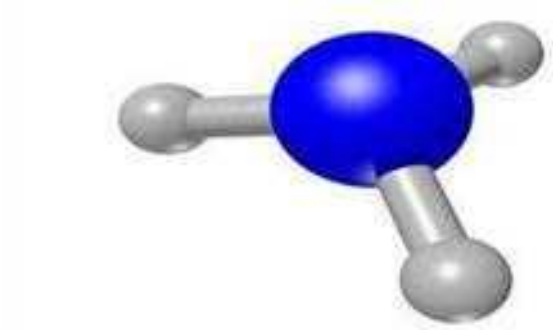
Ammonia is a compound with a chemical formula, NH3. It exists in the water as un-ionized ammonia (NH3) and ionized ammonium (NH4+), and the sum of both parts is the total ammonia nitrogen (TAN). In an aquaponics system, ammonia is primarily produced by fish. Ammonia takes part in the nitrogen cycle because it can be present in the water from uneaten fish food, decomposed organic materials, and fish excretion. Ammonia is toxic in high levels, but it is necessary to give plants the system the nutrients they need.

Ammonia poison in aquaponics fish can lead to:

Damage to fish tissues, especially in gills and kidneys.

- Physiological imbalance
- Impaired fish growth.
- Weak resistance to diseases.
- Death

So in aquaponics, it is essential to monitor the ammonia level in your aquaponics system to ensure you create the best environment for the fish, plants, and bacteria in your system.



Ammonia's role in an Aquaponics System

Ammonia is essential for the nitrogen cycle and is produced when the bacteria in the intestine break down the protein from the food source. The process known as nitrification converts ammonia into nitrite (NO2) by the type of nitrifying bacteria called nitrosomonas. Nitrite is then converted into nitrate (NO3) in the second nitrification step by another nitrifying bacteria called nitrobacter. Nitrate is the form of nitrogen used by plants to grow and survive

Important to Convert Ammonia into Nitrates?

- Ammonia is so harmful to the fish that it may cause their death unless it is converted into nitrates or diluted at a non-toxic level.
- Plants cannot absorb ammonia so. It must be converted into nitrates so that plant roots can absorb the nutrients.

The Relation Between pH and Ammonia

The Relation between pH and ammonia is shown below:

The higher **the** pH levels in the system, the higher the ammonia levels.

- Lower pH levels in the system will also result in lower ammonia levels.

To maximize nitrification efficiency in aquaponics systems, you must aim for low PH level. Nitrification efficiency is often a problem for new aquaponics systems. However, as the system matures and stabilizes, nitrification efficiency can be achieved at lower pH levels. The ideal pH level range for aquaponics is 6-6.4.

How to Test Ammonia Level in an Aquaponics System?

You can test ammonia levels in your aquaponics system by using test stripes avalible online or in your local pet stores. To test, put a sample of your tank water in a clean container and take a test strip and dip it in the water for at least 10 seconds. Take the strip out and shake out the excess water and compare the color to the comparison chart that comes with the test strips.

How to Utilize and Remove Ammonia in Aquaponics Systems

If the ammonia produced by fish accumulates in an aquaponics system, it would soon cause fish death. However, the ammonia in aquaponics systems can be removed by the nitrifying bacteria that converts ammonia into nitrites and then into nitrates by nitrification.

Nitrification performs optimally when the dissolved oxygen levels are high, and the organic matter (produced by uneaten fish food and other wastes) is low. If the oxygen levels are too low, the nitrification process will also slow down or stop, leading to the accumulation of ammonia levels that are toxic to the fish. Nitrite is also harmful to fish at levels of 5 ppm.

Ammonia and nitrite removal in aquaponics systems is called biofiltration. Without a healthy and functioning biofilter, the waste products produced by the fish will accumulate, resulting in inadequate amounts of plant nutrients. If there are insufficient nutrients for the plants, the system will not perform properly.

Ammonia's Role in Establishing a Biofilter

A biofilter is a place where the nitrifying bacteria colonize. A separate biofilter is sometimes unnecessary in the raft and media-based aquaponics systems because the rafts, media, tank, and other surfaces can provide enough area for the bacteria to colonize. However, some aquaponics growers still opted to use biofilters to help break down organic matter and provide more micronutrients and dissolved oxygen in the water. A biofilter is needed in a nutrient film technique (NFT) system because there is not enough surface area for the bacteria to colonize.

Building bacterial colony in a new aquaponics system is known as cycling. Cycling is an essential first step in setting up an aquaponics system. The cycle is not complete until a healthy bacterial colony of nitrifying bacteria is established, and plants will not grow.

Establishing a healthy bacterial colony involves the steady and constant introduction of a source of ammonia into the system, which feeds the new bacterial colony and allows it to grow, thus creating a biofilter. There areseveral methods of cycling a new aquaponics system.

How to Adjust Ammonia in Aquaponics

Too High Ammonia Levels

Even if your aquaponics system is stable, it is a good idea to check the ammonia levels weekly to monitor and catch changes early and make adjustments before they become a problem. Higher ammonia levels occur when more ammonia is produced than can be handled by the biofilters. Possible causes for this are overfeeding of fish, high fish density for the volume of water, or not enough aeration. Below are the methods of adjusting ammonia in aquaponics systems.

You can bring down the ammonia levels in your system by the following:

1. Checking the pumps and DO levels.
2. Adjusting the feeding rates of fish or the fish density (A rule of thumb is per 2 gallons of water 1lb of fish).
3. Increasing nitrification efficiency.

4. Reducing the quantity of nitrogen going into your system by lowering feeding rates, removing dead fish, and removing uneaten fish feed after feeding.

Too Low Ammonia Levels

If your system has not had enough ammonia, your plants will not grow. So enough ammonia must be produced and converted into nitrate for your plants to thrive. Low ammonia occurs when there are too few fish in the system or there is too much water for the number of fish grown. The solution to low ammonia levels is to add more fish to your system, increase feeding rates or use a smaller tank.

Fig 2 :-**Adjust Ammonia in Aquaponics**

Fig 3:- collection of water sample for ammonia test by using siphoning pipe

2.3 Aquaponics

CORVALLIS, Ore. – Skip the soil and try growing vegetables in an aquaponics system that turns fish waste into fertilizer for your plants.

"An aquaponics system grows both fish and plants that can be harvested sustainably," said David Landkamer, an aquaculture specialist with the Oregon State University Sea Grant Extension program. "It's an elegant system."

Here's how it works*:* Fish are typically raised in indoor tanks, troughs or outdoor ponds, where they produce excrement. The water with the waste from the tank flows to a

hydroponics tray where plants grow in the water without soil. The waste is toxic to the fish but is a rich fertilizer for the plants. As the plants absorb the nutrients, the water is purified for the fish. The clean water can then be recycled to the fish tank. Because you can't use pesticides or chemical fertilizers that would harm the fish, it's a natural organic production system. You can grow just about any kind of plant, Landkamer said. Any leafy greens such as lettuce, kale, Swiss chard and arugula are the easiest to cultivate, he said. You can also grow herbs such as basil, mint and chives. Other crops include cucumbers, shallots, snow peas, eggplant, tomatoes, cabbage, cauliflower, peppers, beans, squash, red onions and even potatoes.

Aquaponics, which began in ancient China and Mexico, is gaining popularity around the world as a means of local food production, Landkamer said. He regularly fields questions from people who want to start small-scale, backyard aquaponics operations or even commercial-scale aquaponics farms. It is possible for hobbyists to start out with aquaponics kits available online and at hydroponics supply stores, Landkamer said.

Aquaponics systems feature a fish tank, trough or outdoors pond and a soil-free bed for plants. The fish container can be made out of fiberglass, glass, concrete or plastic. Containers can range in size from a 20 to 40 gallon plastic tote to a large plastic aquarium about 4 to 5 feet deep and 6 to 10 feet wide. Kits are best suited for a temperature-controlled environment in a greenhouse or inside your home, Landkamer said. Fill the plant bed with pebbles made of clay or gravel, or grow the plants on foam or bamboo "rafts" that float on the water, Landkamer said.

Beyond the tank and bed, each system relies on the following customizable components: a solids removal area, a biofiltration system to grow helpful bacteria that break down the fish waste, a water sump and pump, and an aeration system. A water sump is a reservoir in which water is collected, and then returned to the rest of the system using a pump.

Depending on which species of fish you choose, you may need to add a heater of some kind to keep the water temperatures just right for the fish and plants. Aquaponics farmers often use inexpensive heat supplies such as solar greenhouses or hot compost, he said.

"These systems require monitoring to make sure everything is in balance and running smoothly," Landkamer said. "You have to pay attention and see how well the fish are feeding, how well the plants are growing and see whether the water is circulating properly."

Besides fish and plants, the aquaponics system naturally produces one other crop – "good" bacteria that convert the toxic components of fish waste into nutrients that plants can consume. You don't need to add these bacteria, but do get a testing kit to monitor oxygen and nutrient levels in the water, Landkamer said.

Tilapia are the most commonly raised fish in aquaponics systems, Landkamer also recommends catfish, trout, common carp, koi, sunfish, goldfish, barramundi, Murray cod and crayfish. Choose freshwater species, he advised. They can be fed standard diets made for each species and available at feed suppliers or pet stores.

Fig 4:- Nursery Rearing For Aquaponics Cultivation

Day 1

Day 3

Day 6

3. Materials and methods .

Experimental Study on Tilipia fish excreting material as a source of nutritions for aquaponic system was constructed in a greenhouse .somewhere near the Freshwater Hatchery Unit on the campus of Krishna University. The system consists of one culture tank , six hydroponic troughs (growing bed) , one sump system for denitrification unit, one water holding tank and reservoir (pre-aeration). Pipelines made of polyvinyl chloride were installed to connect the culture tank and hydroponic trough to recirculate the water. Layout of prototype of aquaponic recirculating system. 1 fish tank, 2 growing bed, 3 sump, 5 water storage tank, 6 air blower, 7 pre-aeration tank. Desalination and Water Treatment one culture tanks arranged and used in the rearing of Tilapia fish . Tube diffusers, connected to an air blower were installed in the culture tank to supply oxygen for fish culture. Water level in each culture tank was kept at 0.85 m deep to maintain the water volume at 500 L. Water lost through evaporation, transpiration and sludge removal is replenished with water in the pre-aeration tank. In this study the square tank with the conical pattern around the ends of the tank was used in which the perforated sheet will be placed between the tank and conical part to create areas of no turbulence, allowing for more rapid settling of particulate waste . This will allow most of the settleable solids to be concentrated and removed from the tank while most of the water flows out toward the end, into the hydroponic trough by gravity. The water flow rate was controlled by a valve. Three of the hydroponic troughs were planted with water **spinach (Ipomoea aquatica**) **Oryzasativa(Rice plant) , Solanumlycopersicu (tomato) , Amaranthus**to study the effect of using plant on water quality parameters. Hydroponic troughs were filled with gravel the size of 0.5–40 mm and porosity of 0.60. Aquaculture wastewater from the fish tank flowed by gravity into the hydroponic trough and the sump, which is the lowest point in the system. The water was then pumped vertically to the sand filtration tanks for particulate removal. After exiting the sand filter the water went directly to storage tank and was flowed by gravity back to the fish tank. 2.2. Hydraulic condition and culture Five experiments were carried out at different flow rates into recirculating aquaculture system (RAS), each operating for 3–4 weeks in order to relate to nutrients removal, water quality and growth of the plant. Tilapia fish with the same stocking density about 5 kg/m3

were introduced into the culture tank and acclimatized for 1 week after setting the desired tested water flow rate. The feeding rate was adjusted according to the intake rate.

Hydraulic loading rate, which is flow rate (Q) divided by total surface area of the trough. c Hydraulic retention time, which can be computed as (surface area × water depth × porosity of gravel trough/flow rate). around 2–5% of bodyweight/day. The fish were fed with a commercial floating pellet manually twice a day at around 9 am to 5 pm each day. These commercial diets contained around 32% protein and 10% moisture. The food size was adjusted to compensate for changes in fish size. No water discharge or displacement except for replacing water lost through evaporation, transpiration, and sludge removal. Ten percent of fish were taken from the culture tank to measure their length and body weight to estimate the growth rate of the fish. Table 1 illustrates the hydraulic condition for this study. 2.3. Plant growth Approximately 15–20 g of water **spinach (Ipomoea aquatica**) **Oryzasativa(Rice plant) , Solanum lycopersicu (tomato) , Amaranthus** seed was required to completely cover the surface of one trough. The seedlings were placed into the holes evenly spaced 5–8 cm apart and planted with two seeds per hole. The experiment was conducted in triplicate and one trough was utilized as controls and contained gravel only. During the germination period (days 2–4), seed germination and seedling height were observed and recorded daily. A sprinkler was used to irrigate the plant in the trough. Effluent samples were collected from each trough once a week for chemical analyses. During the growth period (days 5–28), the height, leaf length and leaf width of 20 plants in each trough were measured and recorded daily. The plants were harvested at height ranging from 45 to 50 cm. Each growing trough was cleaned and the biomass of plants was measured and recorded. 2.4. Sampling and analysis Water samples were taken once a week from each culture tank, influent and effluent of the hydroponic trough, sump, water holding tank and inflow of culture 22 A. Endut et al. / Desalination and Water Treatment 5 (2009) 19–28 tank. The samples were analyzed for 5-day biochemical oxygen demand (BOD5), total suspended solid (TSS), total ammonium nitrogen (TAN), nitrite nitrogen (NO2 ! -N), nitrate nitrogen (NO3 ! -N) and total phosphorus (TP). Dissolved oxygen, pH, and temperature were also monitored. Weekly sampling was carried out between 8.30 am and 9.30 in each sampling date and refrigerated at 4EC in labeled polythene bottles for chemical analysis. The BOD5 and TSS analyses were

performed according to the Standard Method (APHA 1998). The TAN, NO2 ! -N, NO3 ! -N and TP measurements were performed using Hach DR4000 spectrophotometer according to a salicylate, diazotization, cadmium reduction and ascorbic acid methods, respectively. The DO and pH of the sample were measured using a DO meter YSI 55A and pH cyber scan waterproof respectively

4. Results and discussion

(Table 1) - Water Parameters in Fish tank

TEST PARTICULARS		Day 10		Day 15		Day 20		Day 25		Day 30	
		AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
P.H		7.5	8.0	7.4	7.8	7.8	8.5	8.0	9.0	9.44	9.5
salinity		8		6		4		3		1	
Dissolved Oxygen(ppm)		4.0		4.0		4.2		4.2		4.4	
Alkalinity (ppm)	Total(ppm)	140		130		120		100		92	
	Carbonates (co3)	30		30		32		32		32	
	Bicarbonates (HCo3)	41		45		48		55		59	
Total	Total	250		223		230		210		200	

Hardness (ppm)	Ca Hardness	75	78	80	80	80
	Mg Hardness	120	100	114	118	120
	Ca++	22	25	28	30	32
	Mg++	28	28	28	28	28
	Sodium(Na)	-	-	-	-	-
	Potassium(K)	-	-	-	-	-
Ammonia (ppm)	T Ammonia	0.390	0.367	0.358	0.321	0.391
	U Ammonia	0.369	0.258	0.147	0.159	0.357

Table -2 Water quality parameter of water collection from Hydrophonicspipe

TEST PARTICULARS		Day 10		Day 15		Day 20		Day 25		Day 30	
		AM	**PM**	**AM**	**PM**	**AM**	**PM**	**AM**	**PM**	**AM**	**PM**
P.H		7.0	7.2	7.1	7.3	7.3	7.2	7.3	7.5	7.3	7.5
salinity		3		2		3		2		2	
Dissolved Oxygen(ppm)		4.0		4.0		4.2		4.3		4.5	
Alkalinity (ppm)	Total(ppm)	185		190		180		182		184	
	Carbonates (co3)	-		-		-		-		-	
	Bicarbonates (HCo3)	41		45		48		55		59	
Total Hardness (ppm)	Total	445		450		468		480		500	
	Ca Hardness	80		85		88		92		100	
	Mg Hardness	400		421		403		401		400	

	Ca++	35	35	36	38	40
	Mg++	88	91	93	95	96
	Sodium(Na)	-	-	-	-	-
	Potassium(K)	-	-	-	-	-
Ammonia (ppm)	T Ammonia	0.369	0.321	0.357	0.325	0.327
	U Ammonia	0.126	0.124	0.147	0.254	0.213

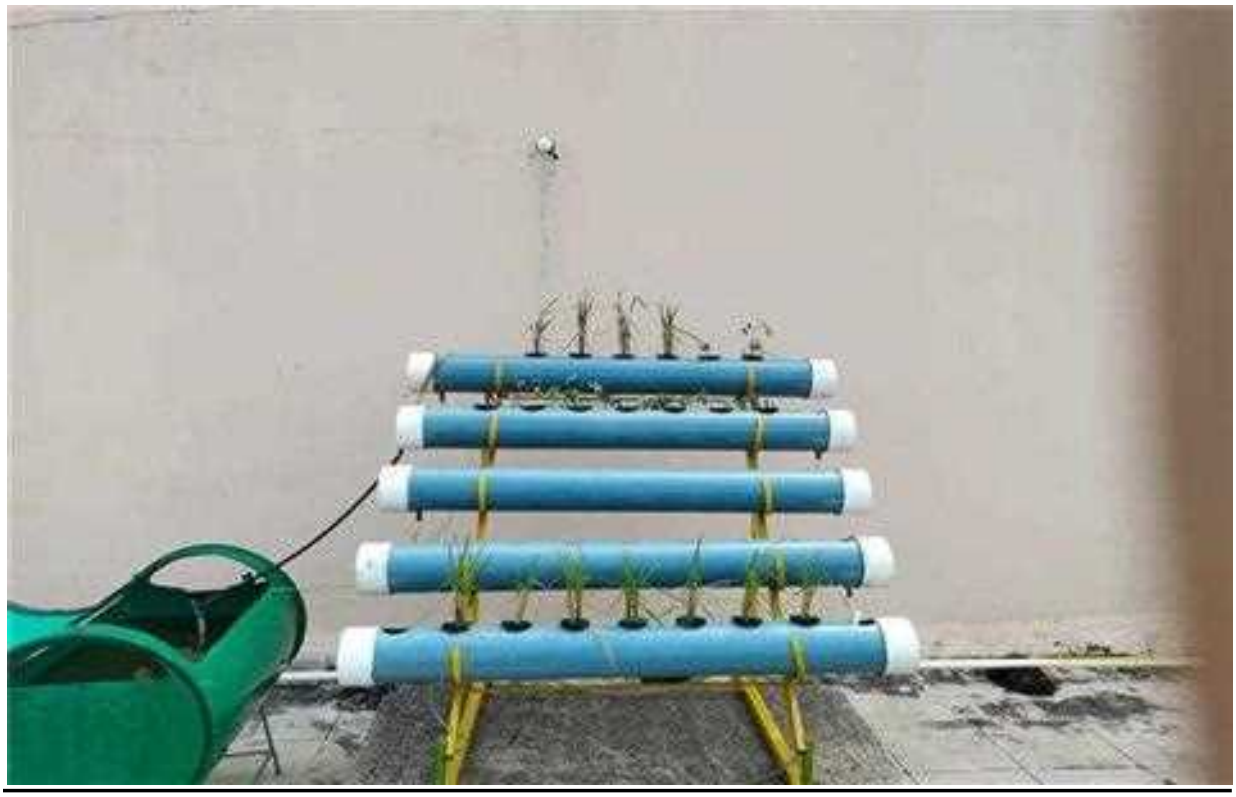


Fig 5:- Aquaponic system

Fig 6 :- Determination of alkalinity and hardness through titration by adding indicators

5. Conclusions

Water spinach (Ipomoea aquatica) **Oryzasativa(Rice plant) , Solanumlycopersicu (tomato) , Amaranthus**grew well and showed a positive response to aquaculture wastewater applications in terms of growth and biomass production. No visual signs of mineral deficiency or disease were noticed. The average plant height at harvest was in the range 45–50 cm and the yield range 2.0–2.2 kg/trough. Plant growth was highest at a water flow rate of 1.6 L/min. The plant was able to significantly reduce the pollution load of the aquaculture wastewater stocked with Tilapia fish. Efficient removal was always achieved in these studies under a wide range of water flow rates because of low pollutant levels of aquaculture wastewater. All flow rates were efficient in nutrient removal and in maintaining the water quality parameters within the acceptable and safe limits for growth and survival of fish. The period of 3– 4 weeks for nutrient removal in this study seemed to be shorter than those that occurred in other system cited from the literature. The reason for this might be that a pilotscaleaquaponic system can develop stable removal processes more quickly than other systems. Because the aquaculture wastewater has low nitrogen concentrations, removal of inorganic nitrogen was extremely efficient under various flow rates trials (0.8–4.0 L/min). The hydroponically grown water spinach was able to significantly reduce the pollution load of the aquaculture wastewater. The BOD5, TSS, TAN, NO2 ! -N, NO3 ! -N and TP reductions were in the range of 47–65%, 67–83%, 64–78%, 68–89%, 42–65% and 43–53% respectively. Results from this study showed that both plant growth and production of Tilapia fish were better at a flow rate of 1.6 L/min.

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EXTRACTION OF CHITIN AND CHITOSAN FROM THE BIOWASTE OF SHRIMP FROM MACHILIPATNAM FISHMARKET

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Dissertation Submitted to

DEPARTMENT OF BIOSCIENCE AND BIOTECHNOLOGY, KRISHNA UNIVERSITY FOR THE PARTIAL FULFILMENT OF PROJECT IN 4TH SEMESTER M.Sc. ZOOLOGY

2023

CERTIFICATE

DECLARATION

The dissertation work entitled "EXTRACTION OF CHITIN AND CHITOSAN FROM BIOWASTE OF SHRIMP FROM THE MACHILIPATNAM FISH MARKET" has been carried out by me in Krishna university campus under the supervision of Dr L.Susheela HOD Dr.Suresh Babu Ch. Asst. Professor and Dr.Swarupa Rani M. Asst. Professor. I declare that the result and contents embodied in this report or original and have no been submitted for project fulfilment in the 4th Semester of M.Sc. Zoology.

PLACE:

DATE:

A. Anjali

SIGNATURE OF THE STUDENT

(A. Anjali)

CERTIFICATE

This is to certify that Miss. A.Anjali. Reg. No. Y20ZOO101001. II year M.Sc. Zoology form Department of Biosciences and Biotechnology. Krishna University Machilipatnam. completed the project entitled as "EXTRACTION OF CHITIN AND CHITOSAN FROM BIOWASTE OF SHRIMP FROM THE MACHILIPATNAM FISH MARKET". entire work has been carried out by her in the Dept. Of BSBT. Krishna university campus from 10th June 2023 to 15th Sep 2023. This work is towards partial fulfillment of Project in the 4th semester of M.Sc. Zoology. These work is original which has not been submitted in part or full for any university/institute.

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EXTERNAL EXAMINAR

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Alisam Anjali

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1. INTRODUCTION

1.1 HISTORY OF CHITIN

1811 Chitin was first discovered by Professor Henri Braconnot, who isolated it from mushrooms andnameit "Fungine"

1823 Antoine Odier found chitin while studying beetle cuticles and named "chitin" after Greek word"chiton"(tunic, envelope)

1838 Cellulose was discovered and noted

1859 Rought discovered chitosan, a derivative of chitin.. 1920s Production of chitin fibers fromdifferentsolvent systems

Home Braco (1786-1956)

1930s Exploration of synthetic fibers

1950s The structure of chitin and chitosan was identified by X-ray

diffraction, infrared spectra, and enzymatic analysis 1970s "Re-discovery" of the interest in chitinandchitosan

• 1977 1" international conference on chitin/chitosan

Chitin and chitosan are valuable, versatile natural materials derived from crustacean exoskeletons. The word "chitin" is retrieved from the Greek etymology, meaning "tunic" or "envelope". Antoine Odier was the first to use the product in 1823.

The name chitin comes in the 1830s, when the substance was isolated in insects. Chitosan was discovered in 1859 by professor C. Rouget. In the 1930s and 1940s, polymers attracts considerable attention as evidenced by about 50 patents secured during that time. Lack of adequate manufacturing facilities and cut- throat competition from synthetic polymer producers restricted commercial development. Revived interest in the 1970s encouraged the need to better utilise shellfish shells. Chitin and chitosan are used in several countries worldwide in a variety of applications, and today there are dozens of applications of chitin and itsderivatives. (Global industry analysis since 2004)

1.2 CHITIN

Chitin is the second-most abundant natural polymer in the world, behind only cellulose. It is also the most abundant naturally occurring polysaccharide that contains amino sugars. This abundance, combined with the specific chemistry of chitin and its derivative chitosan, make for the impressive array of potential applications.

Chitin occurs as a component of crustacean shells, insect exoskeletons, fungal cell walls and plankton. It is found in association with proteins and minerals, such as calcium carbonate. The different sources of chitin differ somewhat in their structure and percentage of chitin content.Chitin ($(C_8H_{13}O_5N)_n$ (/'kaitin/ KY-tin) is a long- chain polymer of N-acetylglucosamine, an amide derivative of glucose. Chitin is probably the second most abundant polysaccharide in nature (behind only cellulose); an estimated 1 billion tons of chitin are produced each year in the biosphere.It is a primary component of cell walls in fungi(especially filamentous and mushroom forming fungi), the exoskeletons of arthropods such as crustaceans and insects,the radulae, cephalopod beaks and gladii of molluscs and in some nematodes and diatoms.

It is also synthesised by at least some fish and lissamphibians. Commercially, chitin is extractedfrom the shells of crabs, shrimps, shellfish and lobsters, which are major by-products of the seafood industry. The structure of chitin is comparable to cellulose, forming crystalline nanofibrils or whiskers. It is functionally comparable to the protein keratin. Chitin has proved useful for several medicinal, industrial and biotechnological purposes.

1.3 CHITOSAN

Chitosan, the unique natural polymer, is commercially derived from chitin. Although typically manufactured by de-acetylating chitin through chemical processes, chitosan is also found in nature, for example as a key component in fungal cell walls.

a chemical substance taken from the shells of sea creatures, that has various uses in industry, farming, and medicine, for example in medical dressings (= coverings for injured skin): The bandage is made with chitosan, a blood-clotting agent found in shrimp shells.

Chitosan is a natural biodegradable polysaccharide extracted from marine natural sources (e.g., crustacean shells). It has been shown to be nontoxic in a range of toxicity tests, both inexperimental animals and humans.

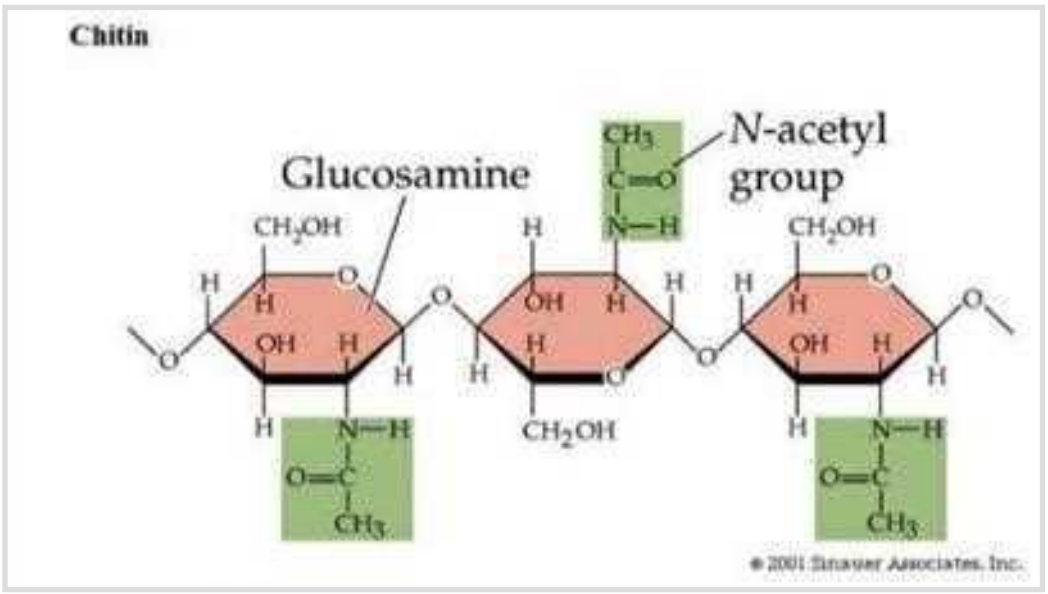

Figure 1: Structure of Chitin

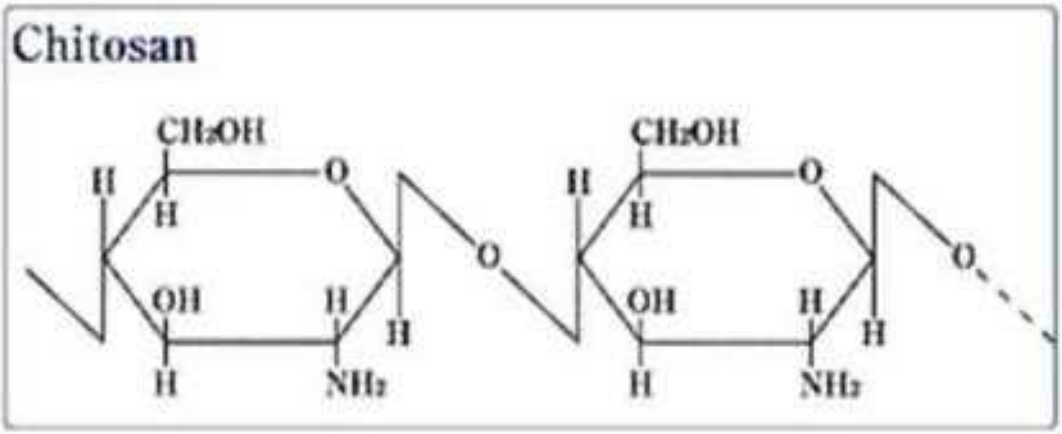

Figure 2: Structure of Chitosan

1.4 SOURCE OF CHITOSAN

→ It is characterized component of the cell walls of fungi, the exoskeleton of arthropods and such ascrustaceans (e.g. crabs, lobster shrimp).

> The backs and internal shells of cephalopods, including squid and octopuses, on the scale and other softtissues of fish and some amphibians.

→ Therefore the major source of chitin/chitosan production in the world is currently from crab and shrimpshells.

1.5 IMPORTANCE OF CHITOSAN

Chitin and chitosan are both biocompatible, biodegradable, and non-toxic biopolymers. They are alsoantimicrobial and hydrating agents

Chitosan exhibits an intrinsic antibacterial activity, inhibiting bacteria and fungi growth. As an example, in Staphylococcus aureus cultures, chitosan treatment promotes structural changes in the so-called membrane-wall complex leading to the impairment of surface cell structures and to bacterial death.

1.6 EXTRACTION AND CHARACTERIZATION OF CHITOSAN FROM SHRIMP

Chitosan is one of the most studied polysaccharides nowadays. Because it is non-toxic, widely used in food, pharmaceutical processes, agricultural, and presents excellent biological properties such as biodegradation in the human body, and antibacterial. In the present study we reported the extraction of lowcost chitosans (Cs1, Cs2, Cs3 and Cs4) from shrimp shells by extraction of chitin (Egypt: case study), then alkaline deacetylation of chitin with strong alkaline solution at different period of time.The different prepared chitosans (Cs1, Cs2, Cs3 and Cs4) were characterized by FTIR spectroscopy, thermal stability, morphology, crystallography, elemental analysis and degree of deacetylation. The data showed that the prepared chitosan Cs2 has the most thermal stability and the highest degree of deacetylation.

A white hard polysaccharide chitin, which known as 2-acetamido-2-deoxy-D-gluco-pyranose units through linkage, is extracted from the crustacean's exoskeletons and also from crabs and shrimps
The alkaline deacetylation of chitin produces a very useful material chitosan, which known as a copolymer of linked 2-amino-2-deoxy-D- gluco-pyranose units, and also it is found naturally in some fungal cell walls.Since it is non-toxic and presents excellent biological properties such as biodegradation in the human body,immunological, antibacterial, and wound-healing activity as shown in chitosan has been widely used in food and pharmaceutical processes and in medical and agricultural drugs . It can be found also in the skeleton of chitosan.

Crab, shrimp and lobster, as well as in the exoskeleton of marine zooplankton spp., including coral and jellyfishes . Also, the chitin can be extracted from various sources to be converted to chitosan by different degree of dacetaylation during using different concentration of NaOH. Due to solubility of chitosan in acidic aqueous medium, various applications at industrial area can be found for it; its solubility is due to the degree of acetylation, molecular weight, and distribution of the acetyl and amino groups along the chain. Also, antimicrobial activity is attributed to chitosan when the amino groups are in cationic form, which means that antimicrobial activity of chitosan is higher at low pH . Chitosan has a broad- spectrum antimicrobial activity against both Gram-positive and Gram- negative bacteria .

1.6.1 MATERIALS

Raw shrimps stated as large size were purchased

1.6.2 METHODS OF EXTRACTION

The extraction of chitosan can be carried out by different four methods under different conditions after removing the loose tissue from the shrimp shells then washed, dried and grind to obtain dry powder. The major procedure for obtaining chitosan is based on the alkaline deacetylation of chitin with strong alkaline solution at different period of time. 1.EXTRACTION METHOD 1

1) Deproteinization process

The deproteinization was occurred by heating of 3 gm of shrimp shells powder after adding 2 N NaOH with ratio of 12ml:1g (w/v) at 70°C for 4 h. The product was neutralized by washing under running tap water. The solid was collected and washed with distilled water. The solid product was dried in vacuum andweighedwith analytical balance.

2) Demineralization process

The dry solid was treated with 10% HCI (3.25 N) with ratio of 14ml:1g (w/v) at room temperature and kept for 4 h. The solid product was collected and washed with distilled water. The solid was then dried.

3) Deacetylation

Then the demineralized product was treated with 35% NaOH (8.75 N) with ratio of 14ml:1g (w/v) at room temperature for 75 h. with stirring. The deacetylated solid was filtered then collected and washed with distilled water. The deacetylated product was dried in a vacuum to give 1.51 gm and then labeled as Cs1.

2.EXTRACTION METHOD 2

1) Demineralization process

The deproteinization process was carried out by weight 3 gm of shrimp shells powder by using 5% NaOH(1.25 N) with a weight to volume ratio of 1g:8ml (w/v). The solution with shrimp shells was refluxed at 70°C for 3 h. The product was collected and washed until clear solution. It was then dried in a vacuum. The product was decolorized with pure acetone for 24 h. The product was collected and washed to neutrality, then dried.

2) Demineralization process

The decolorized product was demineralized by using 1% HCI (0.32 N) with a weight to volume ratio of 1g:10ml for 24 h. at room temperature. The product was collected and washed to give light brown powder.

3) Deacetylation

The N-deacetylation of the demineralized product was carried out by using 55% NaOH (12.5 N) with weightto volume ratio of 1g:5ml at 100°C for 12 h. The product was washed with distilled water and dried to produce 1.69 gm and then labeled as Cs3

2. REVIEW OF LITERATURE

Chitin is a large, structural polysaccharide made from chains of modified glucose. Chitin is found in the exoskeletons of insects, the cell walls of fungi, and certain hard structures in invertebrates and fish. In terms of abundance, chitin is second to only cellulose. In the biosphere, over 1 billion tons of chitin are synthesized each year by organisms. This extremely versatile molecule can form solid structures on its own as in insecChitin, like cellulose and keratin, is a structural polymer. Made from smaller monomers, ormonosaccharides, structural polymers form strong fibers. When secreted inside or outside of cells in an organized way, the fibers form weak bonds between each other. This adds strength to the entire structure. Chitin and cellulose are both made from glucose monomers, while keratin is a fibrous protein. The various structural polymers arose early in the evolution of life, because they are seen only in certain groups. Cellulose is exclusive to plants, keratin to animals, and chitin to the arthropods, mollusks and fungi. Chitin and cellulose both evolved early-on in the history of life, while keratin arose in certain animals long after plants and fungi had branched off from the other eukaryotes.t wings, or can combine with other components like calcium carbonate to make even stronger substances like the shell of a clam.

Like cellulose, no vertebrate animals can digest chitin on their own. Animals that eat a diet of insects often have symbiotic bacteria and protozoa which can break down the fibrous chitin into the glucose molecules that compose it. However, because chitin is a biodegradable molecule that dissolves over time,it is used in a number of industrial applications, such as surgical thread and binders for dyes and glues.

2.1 FUNCTION OF CHITIN

Chitin, like cellulose and keratin, is a structural polymer. Made from smaller monomers, or monosaccharides, structural polymers form strong fibers. When secreted inside or outside of cells in an organized way, the fibers form weak bonds between each other. This adds strength to the entire structure. Chitin and cellulose are both made from glucose monomers, while keratin is a fibrous protein.The various structural polymers arose early in the evolution of life, because they are seen only in certaingroups. Cellulose is exclusive to plants, keratin to animals, and chitin to the arthropods, mollusksand fungi. Chitin and cellulose both evolved early-on in the history of life, while keratin arose in certain animals long after plants and fungi had branched off from the other eukaryotes.

2.2 STRUCTURE OF CHITIN

Chitin is made up of modified glucose monosaccharides. Glucose exists as a ring of carbon and oxygenmolecules. Bonds between glucose molecules are known as glycosidic bonds. The oxygens that typicallyform hydroxyl groups bonded to the carbon ring can also form a bond with another carbon instead of a hydrogen. In this way, monosaccharides can be linked together in long chains. Chitin is formed by a series of glycosidic bonds between substituted glucose molecules.

Chitin is different from cellulose because of the substitution that occurs on the glucose molecule. Insteadof a hydroxyl group (OH), the glucose molecules in chitin have an amyl group attached that consists of carbon and nitrogen. Nitrogen is an electrically positive molecule, while the oxygen double bonded to thegroup is electrically negative. This produces a dipole in the molecule, which increases thehydrogen bonds that can formed between these molecules and the molecules around them. When combined in a matrix with various compounds and other chitin molecules, the resulting structure can bevery hard because of all the weak interactions between nearby molecules.

Processing of chitosan

Chitin can be isolated from crustacean shells by chemical process. It involves some process steps as follows

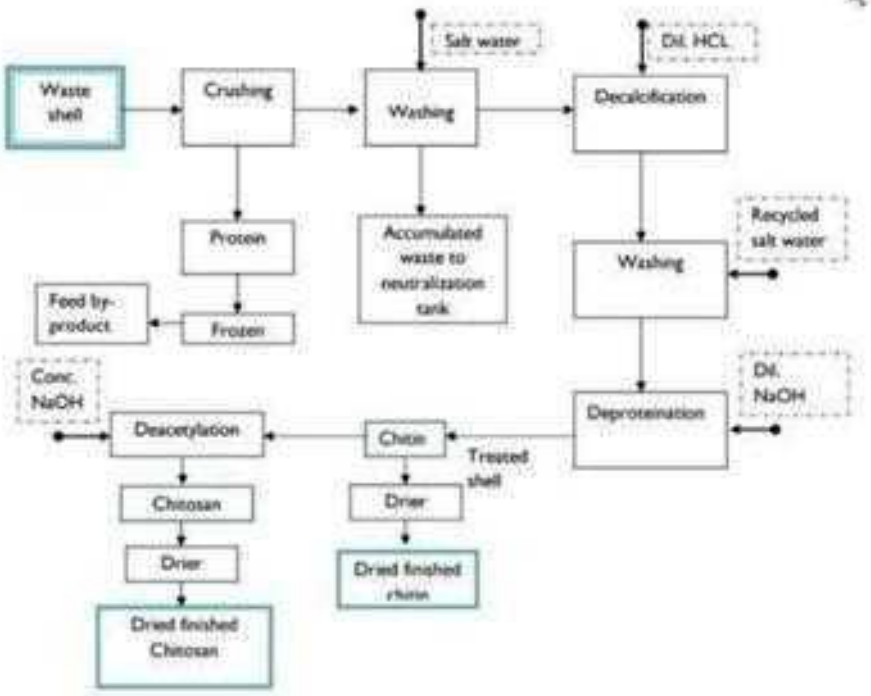

Figure 3. Manufacturing process[7]

2.3 CHITIN IN FUNGI

In fungi, chitin is used to create a cell wall. Much like cellulose in plants, the chitin is deposited extracellularly with proteins and other molecules. This forms a rigid cell wall between cells, which help the organisms retain their shape. Much like in plant cells, water can be retained in the cells to create water pressure against the cell wall. This is known as turgor pressure and adds to the strength of each cell. Fungi are able to push through multiple layers of leaf litter as they grow, which can weigh severalpounds.This comes in part from the strength of chitin as a structural fiber.

2.4 CHITIN IN MOLLUSKS

Chitin is seen in a range of other forms in the mollusks. Chitin is used in both lower mollusks and the more derived cephalopods. In mollusks such as snails, chitin is a part of the radulae, an organ that lookslike a spiked tongue. The mollusks use the radulae to scrape algae and other food from the hard surfaces it grows on. The cephalopods also use chitin, but to form a beak which can be used to bite through the hard shells of their prey items. Ironically, most of the prey items are arthropods, and their shells are also made from chitin.

3. PROPERTIES OF CHITOSAN

Chitosan is the only polycation in nature and its charge density depends on the de- gree of acetylation and pH of the media. The solubility of the polymer depends on the acetylation degree and molecular weight. Chitosan oligomers are soluble over a wide pH range, from acidic to ba- sic ones (i.e., physiological pH 7.4). On the contrary, chitosan samples with higher Mw are only soluble in acidic aqueous media even at high deacetylation degrees. This lack of solubility at neutral and basic pH has hindered the use of chi- tosan in some applications under neutral physiological conditions (i.e., pH 7.4). This is the reason why a great number of chitosan derivatives with enhanced solu- bility have been synthetized.

In 2019, the global chitosan market size was valued at USD 6.8 billion, and it is expected to expand at a revenue based CAGR of 24.7% between 2020 and 2027. The drivers for the market's growth are the increasing application of the polymer in water treatment and several high-value industries such as the pharmaceutical, biomedical, cosmetics and food indus- tries . Some of the interest areas identified include the modification of the polymers to extend their applicability; knowledge of the mechanisms involved in the biological activity of chitosan, chi- tosan derivatives and chitooligosaccha- rides; and the in-depth study of chi- tosanolytic and chitinolytic enzymes pre- sented in different microorganisms

3.1 SOLUBILITY

Chitosan is produced by deacetylation of chitin; in this process, some N-acetylglu- cosamine moieties are converted into glucosamine units. The presence of large amounts of protonated -NH2 groups on the chitosan structure accounts for its solubility in acid aqueous media since its pKa value is approximately 6.5 . When around 50% of all amino groups are protonated, chitosan becomes soluble.

Chitosan solubility depends on different factors such as polymer molecular weight, degree of acetylation, pH, tem- perature, and polymer crystallinity. Homogeneous deacetylation (alkali treat- ment, 0 °C) of chitin permits the produc- tion of polymers soluble in aqueous acetic acid solutions with DD as low as 28%, with this value never being reached under heterogeneous deacetylation (al- kali treatment, high temperatures). Moreover, with a DD of 49%, the sam- ples are soluble in water. This behaviour is explained by the fact that homoge- neous deacetylation leads to an increase in the number of glucosamine units and a modification in the crystalline structure of the polymer. Depending on polymer DD, these modifications range from a re- duction in crystal size and crystal perfec- tion to the presenceof a new crystal structure

close to ß-chitin . Sogias et al. studied the role of crystallinity and inter- or intramolecular forces on chitosan solubility; in this work, a parent chitosan sample was half re-acetylated with anhydride acetic or fully N-deacety- lated under homogeneous conditions. After reacetylation, the solubility of the polymer was expanded until pH 7.4, while a slight reduction in the solubility range of the fully deacetylated chitosan was determined. The lower solubility was explained due to the increase in the poly- mer crystallinity after
deacetylation, which offsets the effect of the increase in glucosamine moieties. On the contrary, a reduction in the crystallinity was ob- served in the half-acetylated sample. The use of hydrogen bond disruptors such as urea or guanidine hydrochloride also al- ters the solubility window of chitosan. In fact, by a combination of chemical and physical disruption of the hydrogen bonds, broad solubility is achieved.

3.2 VISCOSITY

The viscosity of polymers is a parametre of great interest from the technological point of view since highly viscous solutions are difficult to manage. Moreover, viscometry is a powerful tool for determining chitosan’s molecular weight, as it is a simple and rapid method even though it is not an absolute method, therefore requiring the determination of constants that are specific to the solvent. The average molecular weight is determined by the Mark-Houwink-Sakurada equation, which relates this parametre with the intrinsic viscosity:

$$n = KM,\alpha$$

where K and a are constants that must be determined experimentally. Several values of K and a have been reported depending on the solvent composition, pH, and ionic strength . Chitosan viscosity depends on the molecular weight of the polymer and deacetylation degree and decreases as the molecular weight of chitosan is reduced. In fact, viscosity can be used to determine the stability of the polymer in solution, as a reduction is observed during polymer storage due to polymer degradation . Shear viscos- ity increases with chitosan deacetylation degree. The shear viscosity at the same rate was studied in two samples with dif- ferent deacetylation degrees (91% vs. 75%) and represented versus intrinsic viscosity ; it was reported that shear viscosity was larger for those samples with the highest deacetylation degree; when the curves were evaluated, straight lines were observed in both chitosan samples This is explained due to the nature of chitosan, as this polymer is a cationic polyelectrolyte because of the amine protonation in acidic media.

Therefore, the higher the DD, the larger chain expansion is expected, as more glucosamine units are found in the polymer chain, leading to a greater charge density in this sample. In order to modulate chitosan viscosity, the addition of different co-solvents has been evaluated; in this sense, Kassai et al. studied the effect of the addition of isopropanol and ethanol to a chitosan solution in 1% acetic acid, reporting that the presence of the cosolvents decreased the intrinsic viscosity of the polymer.

3.3 CHEMICAL PROPERTIES OF CHITOSAN

✓ Linear aminopolysaccharide with a high nitrogen content

✓ Rigid D-glucosamine structure: hydrophilicity, crystallinity

✓ Weak base (pKa: 6.3). Deprotonated amino group can act as strong nucleophile

✓ Enable to form intermolecular hydrogen bonds: high viscosity

✓ Existence of reactive groups for chemical activation and cross-linking

✓ Insoluble in water and organic solvents, but soluble in dilute aqueous acid solutions.

✓ It forms salts with organic and inorganic acids

✓ Complexing and chelating properties

✓ Ionic conductivity

✓ Polyelectrolytes (at acid pH)

✓ Cationic biopolymer with high charge density (one positive charge per glucosamine residue)

✓ Flocculating agent (interacts with negatively charged molecules)

✓ Entrapment and adsorption properties (filtration and separation)

✓ Film-forming ability (adhesive materials for isolation of biomolecules)

The reactive groups found in chitosan are a primary amino group (C2) and primary and secondary hydroxyl groups (C6, C3). Glycosidic bonds and the acetamide group can also be considered functional groups. These functional groups allow for a great number of modifications, producing polymers with new properties and behaviours.

Chitosan derivatives have been produced, aiming to improve chitosan's properties, such as solubility or biodegradability, or to introduce new functions or properties. For instance, solubility has been improved in water aqueous media by deacetylation, depolymerization, or quaternisation among other processes . New chitosan activ- ities have been reported after its modification, for example, 6-O-sulphated chi- tosan promotes neuronal differentiation while phosphorylated chitosan inhibits corrosion.

The field of chitosan chemistry is wide, and in this review, we want to focus on two types of processes, chitosan phosphorylation and chitosan degradation. Our group has participated in the development of a phosphorylated derivative via a simple method in which chitosan and phosphorus acid are mixed at the same ratio and formaldehyde is added at 70 °C.

This N-methylene phosphonic chitosan is soluble in water and keeps the filmogenic properties of the parent chitosan. With a similar methodology, a soluble in water N-methylenephenyl phosphonic chitosan has been produced . Additionally, the surfactant derivative N-lauryl-N-methylene phosphonic chitosan was produced via N-alkylation of N-methylene phosphonic chitosan . This deriva- tive has a lower solubility in aqueous media compared to N-methylene phosphonic chitosan but better solubility in organic media and forms micelles. N-methylene phosphonic N-methylene carboxylic chitosan has been obtained in water- soluble form using N-methylene phosphonic chitosan and glyoxylic acid. The polymer maintains the filmogenic properties of parent chitosan and, because of the presence of multidentate ligands, its use as abivalent metal chelating agent is proposed .

Although the use of chitosan as a gene carrier has been reported, the use of this biopolymer for this application is limited due to a relatively low transgenic efficacy. Phosphorylated derivatives have shown better performance (transfection was improved 100-fold) and therefore are more suitable than chitosan to this end. Moreover, phosphorylated derivatives also exhibit and improve metal ion chelating activity when compared to the parent chitosan .

Due to the presence of cleavage glycosidic bonds, it is possible to degrade chitosan, thus reducing its molecular weight. As previously mentioned, the control of chitosan depolymerization (polymer size) permits us to control some properties such as solubility or vis- cosity. Moreover, the biological and technological properties of chitosan are related to size, among other properties as previously reviewed . Chitosan degradation can occur through different mechanisms such as acid hydrolysis, oxidative-reductiveor nitrous acid depolymerization, ultrasonic degradation, or enzymatic degradation using specific and non- specific enzymes. Chitosan has four types of glycosidic linkages -D-D-, -A- A-, -A-D- and -D-A- (where A and D denote N-acetylglucosamine and glucosamine monomers, respectively). Depending on the process, there

is a prevalence in the breakage of certain linkages and therefore different samples can be produced from the same parent chitosan by selecting different methodologies. Chemical and physical methods are less selective than enzymatic ones for producing specific patterns due to enzyme-specific recognition but by con- trolling the parametres of the process some control over the composition can be gained.

3.4 BIOLOGICAL PROPERTIES OF CHITOSAN

✓ Non-toxity

✓ Biodegradality

✓ Biocompatibility

✓ Citocompatibility

✓ Antimicrobial activity

✓ Anticholesterolemic activity

✓ Antioxidant activity

✓ Anti-inflamatory action

✓ Analgestic action

✓ Haemostatic action

✓ Mucoadhesion

✓ Anginogenesis stimulation

✓ Macrophage activation

✓ Granulation and scar formation

✓ Adsorption enhancer

Besides the various chemical properties, chitosan shows diverse biological properties which are summarized as follows:

1. Biocompatible:

   i. Natural polymer ii. Safe and non-toxic
   iii.Biodegradable to normal body constituents

2. Binds to mammalian and microbial cells aggressively

3. Regenerative effect on connective gum tissue

4. Accelerates the formation of osteoblast responsible for bone formation

5. Haemostatic (causes stop bleeding)

6. Fungistatic (inhibiting the growth of fungi)

7. Spermicidal (birth control)

8. Antitumor or Anticancer (inhibiting the growth of tumor or cells)

9. Anticholesteremic (cholesterol lowering agent)

10. Central nervous system depressant (slow down the brain activity)

11. Immunoadjuvant (involved in the improvement of immune response)

4. MATIRIAL METHOD

4.1 INTRODUCTION

Chitosan is one of the most studied polysaccharides nowadays. Because it is non-toxic, widely used in food, pharmaceutical processes, agricultural, and presents excellent biological properties such as biodegradation in the human body, and antibacterial. In the present study we reported the extraction of low cost chitosans from shrimp shells by extraction of chitin (then alkaline deacetylation of chitin with strong alkaline solution at different period of time.
A white hard polysaccharide chitin, which known as 2-acetamido-2-deoxy-D-gluco-pyranose units through linkage, is extracted from the crustacean's exoskeletons and also from crabs and shrimps
The alkaline deacetylation of chitin produces a very useful material chitosan, which known as a copolymer of linked 2-amino-2-deoxy-D- gluco-pyranose units, and also it is found naturally in some fungal cell walls.Since it is non-toxic and presents excellent biological properties such as biodegradation in the human body,immunological, antibacterial, and wound-healing activity , chitosan has been widely used in food and pharmaceutical processes and in medical and agricultural drugs. It can be found also in the skeleton of chitosan.

Crab, shrimp and lobster, as well as in the exoskeleton of marine zooplankton spp., including coral and jellyfishes [10]. Also, the chitin can be extracted from various sources to be converted to chitosan by different degree of dacetaylation during using different concentration of NaOH [11]. Due to solubility of chitosan in acidic aqueous medium, various applications at industrial area can be found for it; its solubility is due to the degree of acetylation, molecular weight, and distribution of the acetyl and amino groups along the chain. Also, antimicrobial activity is attributed to chitosan when the amino groups are in cationic form, which means that antimicrobial activity of chitosan is higher at low pH. Chitosan has a broad-spectrum antimicrobial activity against both Gram-positive and Gram- negative bacteria .

4.2 MATERIALS AND METHODS

4.2.1 APPARATUS

- ✓ Weighing balance
- ✓ Mixer
- ✓ Thermometer
- ✓ Burner
- ✓ Filters and filterpapers
- ✓ Butter papers
- ✓ Beakers and flasks
- ✓ Volumetric flask
- ✓ Pipette
- ✓ Dropper
- ✓ Stirrer
- ✓ Oven
- ✓ Measuring jars
- ✓ Biowaste

4.2.2 CHEMICALS

1. 2.Normality
NaoH2. 10%
Hcl[3.25N]
3. 35% NaoH [8.75N]

4.3 PROCEDURE OF EXTRACTION

4.3.1 Methods (Extraction of Chitosan)

The extraction of chitosan can be carried out by different four methods under different conditions after removing the loose tissue from the shrimp shells then washed, dried and grind to obtain dry powder. The major procedure for obtaining chitosan is based on the alkaline deacetylation of chitin with strong alkaline solution at different period of time.

4.3.2 Extraction Method

1) Deproteinization process

The deproteinization was occurred by heating of 3 gm of shrimp shells powder after adding 2 N NaOH with ratio of 12ml:1g (w/v) at 70°C for 4 h. The product was neutralized by washing under running tap water. The solid was collected and washed with distilled water. The solid product was dried in vacuum andweighedwith analytical balance.

2) Demineralization process

The dry solid was treated with 10% HCl (3.25 N) with ratio of 14ml:1g (w/v) at room temperature and kept for 4 h. The solid product was collected and washed with distilled water. The solid was then dried.

3) Deacetylation

Then the demineralized product was treated with 35% NaOH (8.75 N) with ratio of 14ml:1g (w/v) at room temperature for 75 h. with stirring. The deacetylated solid was filtered then collected and washed with distilled water. The deacetylated product was dried in a vacuum to give 1.51 gm and then labeled as Cs1.

4.3.3 Extraction Method

1) Deproteinizationprocess

The deproteinization process was carried out by weight 3 gm of shrimp shells powder by using 5% NaOH(1.25 N) with a weight to volume ratio of 1g:8ml (w/v). The solution with shrimp shells was refluxed at 70°C for 3 h. The product was collected and washed until clear solution. It was then dried in a vacuum. The product was decolorized with pure acetone for 24 h. The product was collected and washed to neutrality, then dried.

1) Demineralization process

The decolorized product was demineralized by using 1% HCl (0.32 N) with a weight to volume ratio of 1g:10ml for 24 h. at room temperature. The product was collected and washed to give light brown powder.

2) Deacetylation

The N-deacetylation of the demineralized product was carried out by using 55% NaOH (12.5 N) with weight to volume ratio of 1g:5ml at 100°C for 12 h. The product was washed with distilled water and dried to produce 1.69 gm and then labeled as Cs3.

4.4 PREPARATION OF REAGENTS

Figure 4: Preparation of reagent 1

Figure 5: Preparation of reagent 2

A. 2N. NaoH

To prapare 1N NaoH we have to dissolve 4.0 gm NaoH to 100ml of distilled water. For 2N NaoH we have to dissolve 8 gm NaoH to 100ml of distilled water

1N NaoH = 4.0 gm NaoH to 100ml distilled water2N NaoH = 8.0 gm NaoH to 100ml distilled water2N NaoH = ? to 1000 ml distilled water

= 80 gm to 1000 ml of

distilledwaterB. 10% Hcl [3.25 N]

Available Hcl is ----35 - 37 %
100 ml - 37%
50 ml -18.5%
25 ml-9.25%
23.8 ml-10% Hcl
So, dilute it to 100 ml water
= 24 ml of Hcl dilute 100 ml of distilledwater

C. 35% NaoH [8.75N]

350 gm NaoH dilute to 1000 ml
distilledwater350 gm = 1000 ml
175 gm = 500 ml

4.5 EXPERIMENTAL PROCEDURE

STEP 1. COLLECTION OF BIOWASTE MATERIAL OF SHRIMP

We have collected the biowatse material of shrimp from the machilipatnam fish market. And brought it to the laboratory for further work progression. Collected material is washed thoroughly with the tap water for the removal of external unwanted material from the shrimp shells. Later the washed material is sun dried with proper precautions

Figure 6: BIowaste Drying

STEP.2 HOMOGENISATION

Figure 7: Homogenised Biowaste Powder

The dried biowaste made in to powder with the help of motor and pistil. Prepares powder stored properly in a closed jar to avoid moisture and it is weighed.

STEP.3 DEPROTINIZATION

The first chemical treatment done by using prepared reagent 2N. NaoH

- ✓ First we have weighed 50 GM's of powdered dry biowaste material on weighing balance.
- ✓ And we have taken 600 ml of 2N. NaoH reagent according to ratio we have taken i.e.,12 ml = 1 gm

  ? = 50 gm

  = 12 × 50 gm

  = 600 ml
- ✓ Now 50 GM's of power added to 600 ml of 2N.NaoH in a large glass beaker
- ✓Glass Beaker is kept in a waterbath in the electric stove. Now the temperature of boiling is adjusted to70°C.
- ✓ It was cooked for 4 hours with frequent stirring and temperature adjustment.
- ✓ Later it was leaved for half an hour to cool., Now the material is filtered by using a filter funnel to extract the deprotinized content.
- ✓ Filtered material is now washed thrice with the distilled water / purified water.
- ✓ Now the obtained content i.e., deprotinized biowaste material is sundried.Product--- deprotinized biowaste solid.

Figure 8 and 9 : Process of Deprotinization

STEP.4 DEMINERALISATION

• To prepare the demineralised product, we have to do collected the deprotinized dry powder which obtained after the deprotinisation
• The power material is soaked in the 10% Hcl reagent, and the measurements we have takenare,14 ml- 1 gm
• we have obtained 20 gm of dry powder,so,1 gm = 14 ml
20 gm =?
=14×20 gm
= 280 ml
• So, we have taken 280 ml of 10% Hcl reagent and dry powder material is added to it. And leave it for4hours at the room temperature.
• product --- demineralised and deprotinized chitin powder
• And it was filtered washed and dried.

Figure 10 and 11 : Filteration and Washing the Chitin Material

STEP.5. DEACETYLATION

• The obtained product from the above steps is protien and mineral less chitin dry powder.
• It weighs about 7 gms.
• So, according to the given ratio

14 ml= 1 gm
? = 7 gm
= 14×7
= 98 ml

• So we have taken 98 ml of 35% NaoH reagent and added 7 gms of dry chitin powder
• It was kept for 75 hours at room temperature later it was filtered and washed thoroughly and dried.

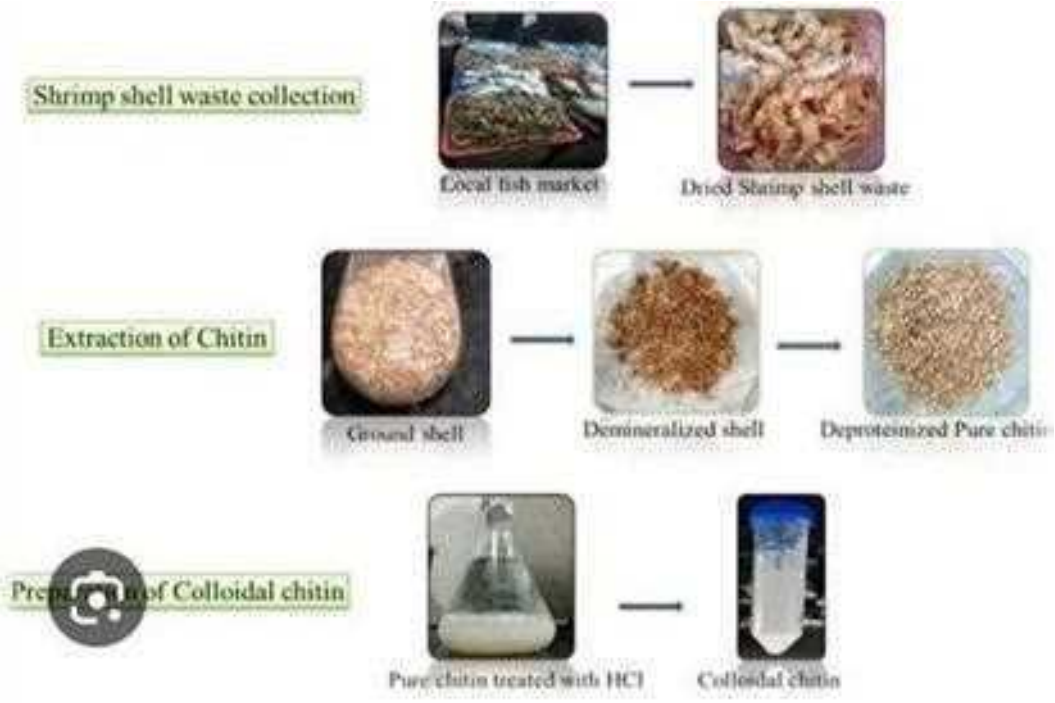

Figure 12: Overall Steps of Chitin and Chitosan Extraction

RESULT

From the above all steps the final obtained product is chitosan.

5. USES AND APPLICATIONS OF CHITOSAN

5.1 USES OF CHITOSAN

Chitosan is a natural cationic polymer that is effective in the treatment of obesity, high cholesterol and Crohn's disease. In addition, Chitosan has the ability to regenerate and promote new tissue formation when injured, burned,...

Chitosan is a cationic polymer obtained from the hard outer skeleton of shellfish, including crabs,lobsters,and shrimp. Chitosan is used for medicinal purposes.

Chitosan is used to treat Crohn's disease, obesity, high blood cholesterol. This preparation is also usedto treat complications commonly experienced by patients with kidney failure on dialysis, including high cholesterol, anemia,weakness, poor appetite, andinsomnia.

Some people apply Chitosan directly to their gums to treat gingivitis that can lead to tooth loss(periodontitis), or chew gum containing Chitosan to prevent tooth decay.

In tissue graft surgeries, plastic surgeons sometimes apply Chitosan directly to places where they have already taken tissue for use else where because Chitosan has the ability to promote new tissue formation.

In pharmaceutical manufacturing, Chitosan is used as a filler in tablets; as a carrier in controlled release drugs to improve drug solubility as well as to reduce bitterness in oral solutions.

5.2 DOSAGE AND HOW TO UES CHITOSAN

Chitosan has been used in clinical studies for cholesterol reduction and weight loss at doses ranging from 0.24g to 15g per day (mean 3.7g/day) for 4 to 24 weeks. Chitosan has been used in patients with kidney failure requiring long-term hemodialysis without any obvious side effects. Chitosan has also been used in prediabetes to control blood sugar at a dose of 1,500 mg/day. A 0.1% Chitosan solution has been used in ophthalmology while a 1% Chitosan solution is used as a mouthwash. With the preparation Chitosan 2%, the patient can use it to apply to the injured skin when needed. Clean hands and injured skin before applying the medicine, apply the product and massage the skin gently afterwards. If you miss adose, take it as soon as you remember. If that time is close to the next use, skip it. Do not take a double dose of the usual dose to make up for a missed dose.

When an overdose is suspected, the patient should be taken to the nearest emergency center promptly for treatment. Bring all medications that the patient took before, including prescription drugs, over-the-counter drugs, health care products, ... for an accurate diagnosis.

5.2.1 UNDESIRABLE EFFECTS

Chitosan is possibly safe for most people when taken by mouth for up to six months or when applied to the skin. When taken by mouth, this preparation may cause constipation, bloating, or mild stomach upset.

However, to be safe, you need to contact your doctor when any unusual symptoms appear during the use of Chitosan preparations.

5.2.2 DRUG INTERACTIONS

When treating with many drugs, competition or synergies between drugs or between drugs and foods and beverages may be caused. As a result, bioavailability, efficacy, and toxicity are altered. Therefore, you need to list and inform your doctor about all medicines and health protection products you are taking or have recently stopped using. Specifically, there is some concern that taking Chitosan weight increase the blood-thinning of increasing the chance of bruis bleeding. So if you are taking Warfarin, avoid Chitosan.

Use of Chitosan in pregnancy and lactation: There is not enough evidence on the safety of Chitosan use during pregnancy and lactation. Therefore, Chitosan should not be used during this period. Shellfish allergy:Chitosan is obtained from the exoskeleton of shellfish. There is some concern that people who areallergic to shellfish may also be allergic to Chitosan. However, people who are allergic to shellfish are allergic to their meat, not the shell. As a result, some experts suggest that Chitosan may not be a problem for people with shellfish allergies. Tell your doctor if you have any of the following conditions: Stomach problems, other blood sugar problems, upset stomach, stomach cramps... Also, tell your doctor if you are pregnant, plan to become pregnant or are breast- feeding. Note: Chitosan is a functional food and does notreplace medicine.

5.3 APPLICATION OF CHITOSAN

5 . 3 . 1 W A S T E W A T E R

TREATMENT

The use of natural additives that are biocompatible, are biodegradable, have low toxicity and are from renewable resources attracted attention of many researchers due to their high ability to retain different pollutants from wastewaters. In this context, there are many research studies that highlight the biosorbent ability of chitosan and their composites for the pollutants from wastewaters such as heavy metal ions, organochloride pesticides, suspended solids, turbidity, organic oxidised substances, fatty and oil impurities or textile wastewater dyes. Furthermore, the increase of adsorption ability of chitosan by chemical modifications leading to the formation of chitosan derivatives, grafting chitosan and chitosan composites gained much attention, being extensively studied and widely reported in the literature. In this chapter the research studies regarding the chitosan application in wastewater treatments as well as the preliminary results on its chemical modification to obtain and utilisation of zeolite-chitosan composites in adsorption of organic pollutants from industrial wastewaters are presented.

Different wastewater decontamination methods that include chemical precipitation, nanofiltration, solvent extraction, ion exchange, reverse osmosis and adsorption have been extensively studied. Out of these methods, adsorption is particularly attracting scientific focus mainly because of its high efficiency, low cost and easy handling and high availability of different adsorbents .

Chitosan is a versatile polysaccharide widely distributed in nature (second most abundant biopolymers after cellulose) produced by alkaline N- deacetylation of chitin. Many application fields are described in scientific publications regarding the use of chitin, chitosan and their derivatives. Wastewater treatment using chitin or chitosan is an important application. According to this, there are many research studies thathighlight the biosorbent ability of chitosan and
their composites to remove the pollutants from wastewater. They could be used as coagulating/ flocculating agents for polluted wastewaters in heavy metal or metalloid adsorption (Cu(II), Cd(II), Pb(II), Fe(III), Zn(II),Cr(III), etc.) for the removal of dyes from industrial wastewater (i.e. textile wastewaters) , as well as for the removal of other organic pollutants such as organochloride pesticides, organic oxidised or fatty and oil impurities.

Due to the high performances, chitosan derivatives are used as adsorption additives in many research investigations. Some examples are derivatives that contain heteroatoms based on nitrogen, phosphorous and sulphur or complex combinations of chitosan with ethylenediaminetetraacetic acid (EDTA) and diethylenetriaminepentaacetic acid (DTPA).

The chitosan composites have been tested in wastewater treatments for adsorption of dyes and heavy metals . To form composites with chitosan, different substances have been used, such as montmorillonite, polyurethane, activated clay, bentonite, zeolites, oil palm ash, calcium alginate, polyvinyl alcohol, cellulose ,magnetite, sand, cotton fibres, perlite and ceramic alumina .

This chapter highlights the application of chitosan and their composites (zeolite- chitosan composites) as adsorbents and flocculants in wastewater treatments including the method of preparation, mechanisms/ kinetics and factors that can affect their efficiency in the pollutant adsorption capacity (pH, biosorbent dosage, contact time). Some experimental results obtained on static adsorption methods applied on industrial and municipal wastewaters are presented.

5.3.2 FOOD INDUSTRY

Chitosan is a collective name used for a group of compounds having various molecular weights, which are produced from chitin by partially or fully de-acetylating and is prepared of β 1,4-linked glucosamine, and itis in deacetylated form of chitin acquired from fungi and/or crustaceans. Due its hydrophilic, cationic and biodegradable nature, chitosan has been cared for a biomaterial, medical, pharmaceutical, drug efficiency, textile, agricultural, food additive for preserving, wastewater clarification, plant pesticide agents and in wound healing. As a compound obtained using various methods, the most prominent features of chitosan are attributable to its antimicrobial and antioxidant properties. Among all the antibacterial compounds fromcrustaceans, chitosan and its derivatives have been widely used for providing the safety of the foods (especially marine based foods) and shelf life extension. This study presents information about antibacterial activity of chitosan, its mode of action against microorganisms, factors affecting its antimicrobial property and its application in food industry and for public health.

Be applied by spraying solutions or dip coating

Allow film-forming due to its long chemical structure

Provide antimicrobial activity, eliminating the use of toxic chemicals

Extend shelf life while maintaining or improving the sensory quality of many foods

Chitosan can be formulated into a number of different formats , including fibres, films, gels, beads or nanoparticles, to create coating and packaging materials that are biocompatible and biodegradable. This means chitosan can replace non-biodegradable and non-renewable polymers that have traditionally been used for these purposes.

Furthermore, chitosan-based matrices have been used for active and intelligent packaging for beverages, such as dairy-based drinks, alcoholic beverages, juices, tea, coffee and fruit juice concentrates.

Chitosan and its derivatives, like chitosan powder, has proven effective in the animal nutrition industry as well. In recent years, recycling protein from biowaste and turning it into domestic animal feed has become a growing market. Chitosan works as an ingredient for animal feed thanks to its prebiotic and antimicrobialeffects, and its nutritional properties.

It has the ability to fortify the gut with beneficial bacteria and suppress pathogenic bacteria. Plus, as chitosan regulates blood cholesterol levels and alleviates fatty liver syndrome, overall animal health increases and producer productivity is enhanced. Chitosan animal feed has been effectively used for pigs,ducks, broilersand laying hens.

5.3.3 DRUG DELIVERY

The aim of this review is to provide an overview of the delivery systems of chitosan-based chemotherapyagents that have been developed for the treatment of tumor cancer. Cancer treatment is a challenge that

has always provided opportunities in different areas of study, due to its very complexity. Innovative options to make chemotherapy an effective treatment by targeting drugs to cancer cells through different modifications in delivery systems are being investigated. Chitosan, a biopolymer that is obtained from the partial deacetylation of chitin (the second most abundant biopolymer on earth) and is present in the

exoskeleton of crustaceans, some insects, and also in the cell wall of some fungi. Chitosan has specific characteristics of solubility, functional groups in its structure, crosslinking power, affinity with other materials, biocompatibility, biodegradability, muco adhesiveness, provides bioavailability of the chemotherapeutic agent on cancer cells, without harming healthy cells. This document compiles some interesting studies on the use of chitosan in conjugation with other agents and safe materials for use in biomedicine, for the design, characterization, and development of new transport systems for chemotherapeutic agents, increasing the efficacy of this therapy in cancer treatment tumors.

Currently, the WHO defines cancer as a generic term for a broad group of diseases that can affect any part of the body, also called "tumors or malignant neoplasms" . According to projection data from the GLOBALCANCER OBSERVATORY (GCO) of WHO, in 2040, about 30.2 million people will suffer from cancer in the world, regardless of gender or age.

Tumor cancer can begin in the cells of any organ of the body, the most common being: colon and rectal cancer, endometrial cancer, liver cancer, pancreatic cancer, prostate cancer, lung cancer, kidney cancer, breast cancer, thyroid cancer, bladder cancer, among others, according to the National Cancer Institute of the United States . The travel of these cancer cells from these organs to other parts of the body and contamination of other tissues is called metastasis. To name it, it always refers to the tumor cells in the initial organ; for example breast cancer with lung metastases.

In general, the treatments carried out for the therapy of carcinoma tumors are surgery (recessions of tumorsattached to organs or parts of organs and tissues already affected), radiotherapy, chemotherapy, targeted therapy, immunotherapy, hormone therapy, and clinical trials. Recent studies point to the visualization of new alternatives for the treatment of tumor cancer. E. dos Santos et al, in their review, found that some epigenetic modulation actions of the tumor microenvironment in head and neck cancer can control tumor maintenance and metastasis . D. Ribatti et al, investigated the controversial role of mast cells in breast cancer progression and angiogenesis . On the other hand, Y. Deng et al studied the effect of biofilms (bacteria) on tumor progression; in a multi-fluidic model (anti-biofilm-antibiotic-drug) against cancer, to simultaneously eradicate biofilms and tumors . Another interesting study is that of S. Mukhopadhyay et al,who focused on the biochemical aspect of cancer and recent advances in autophagy signaling in the tumormicroenvironment, to be used as a possible cancer therapy . New research within invivo studies (rats), carried out in India by N. Subhapradha et al, indicates that chitosan from squid showed antioxidant and antilipidemic properties that can protect liver cells from cancer-produced by N- diethylnitrosamine molecules
.

While all these new approaches to cancer treatment research are being developed, existing therapies are also being refined to improve their efficacy. One of the therapies considered effective, but not selective, is chemotherapy. Based on drugs to disrupt the formation of cancer cells (destroying them or preventing their multiplication), they enter the bloodstream and travel throughout the body, also affecting rapidly dividing healthy cells, generating systemic toxicity . The mechanism of action of some chemotherapeutic agents is growth factor inhibition, angiogenesis inhibition, DNA synthesis inhibitors, among others. There are severalforms of chemotherapy administration: intravenous, oral, injected, intra-arterial, injected into the peritoneum, or topical . However, being unable to differentiate healthy cells from cancerous cells, the chemo pharmaceutical can affect healthy cells and generate unpleasant and often serious side effects in patients. It is well known that the application and dosage of drugs alone or in combination with other drugs depend on the stage of cancer and the patient's health status. In addition, there are generalized side effects for certain drugs, and many times these can vary among patients receiving the same drug.

5.3.4 COSMETICS

Chitosan and its derivatives, such as chitosan powder, can be used in aqueous solutions or solid form, andit can be combined with other hydrating agents, solar filters and other bioactive products, facilitating their effects. Chitosan is biocompatible, making it well suited for skin use and compatible with other ingredients, such as glucose, oils, fats, acids, and more. It is a highly effective hydrating agent with film-forming abilities,simultaneously supplying water while preventing dehydration. Chitosan is multifunctional and can greatly contribute to personal care products by replacing undesirable chemicals, while it also enhances the permeability of other active ingredients.

Chitosan is commonly used in cosmetic and skin care applications as it helps to maintain skin moisture, tone skin, treat acne, provide extracellular matrix support, and promote the skin's natural barrier function. Chitosan is a natural stimulating agent in the process of skin regeneration and wound healing, promoting proper histoarchitectural tissue organization with optimal collagen structure, making it an ideal ingredient for anti-aging skincare products and wound healing.

Chitosan is a bioactive polymer that is of great interest to the cosmetic, hygiene, personal care and cosmeceutical industry.

With multiple applications across multiple industries, pure chitosan has great potential, including in the field of cosmetics and cosmeceuticals. Cosmeceuticals, derived from the words ‘cosmetic and pharmaceutical,'have drug-like benefits and contain active ingredients, contributing to beneficial effects onhuman health.

5.3.5 ANTI BACTERIAL ACTIVITIES

Injection of pathogens and disruption of normal microbiota cause enteric infections . Enteric infections mainly manifest as fairly distinct clinical syndromes, including acute vomiting, acute watery diarrhea, profuse watery diarrhea, invasive or bloody diarrhea (dysentery), persistent diarrhea, and enteric fever. Enteric infections are induced by viruses, bacteria, protozoa, or parasites, e.g., norovirus, Shigella spp., Vibrio cholerae, Listeria, Shiga toxin-producing Escherichia coli (STEC), Clostridium difficile, Salmonella typhimurium, and Giardia lamblia . Pathogens contain virulence factors such as enterotoxins and flagella, which increase intestinal intracellular cyclic nucleotides and activate Cl^- channels in the apical membrane of enterocytes, resulting in increased fluid secretion and decreased fluid absorption [6]. Such a mechanism explains the cause of microbial diarrhea.

Rehydration is the backbone of the treatment of enteric infections. Numerous cases show that oral rehydration salts (ORS) can effectively rehydrate patients . Severe sufferers require intravenous fluids and anti microbial therapy . Antibiotics demonstrated benefits in randomized controlled trials (RCT) inthe treatment of infection with Shigella , Vibrio cholerae , and Enterotoxigenic E. coli (ETEC) , especially in moderate or severe cases. Recently, there is a consensus that antibiotics overuse contributed to increased drug resistance and the resulting dysbiosis. Novel antibiotics are massively produced, but the continuous fear of the resulting antibiotic resistance and dysbiosis elicited researchers to concentrate on the application of nonantibiotic compounds as antimicrobial agents .

Figure 13: Applications of Chitosan

6. ANALYSIS AND CONCLUSION

Chitin and chitosan are attracting a great deal because of their distinctive biological and physico-chemical characteristics. Chitin and chitosan have been used in various industries ranging from waste management to aqua food processing, medicine and Biotechnology. To date a lot of research been done to produce chitin and chitosan from various sources like shrimp and other sources like insects and moluscus.

The fresh water prawn Macrobrachium rosenbergii and shrimp Peneaus monodon are one of the potential species for chitosan production. In the present study biowaste of 580mg M.rosenbergii goes to 32.44gms chitosan, P.monodon produced 455 gms biowaste and this biowaste produced 26.64gms of chitosin.

Extraction of chitosan from freshwater prawn Macrobrachium rosenbergii and tiger shrimp Penaeus monodon exoskeleton requires chemical treatment the shell even though contains majority of chitin, also has proteins and minerals. The chitin and chitosan are used in the preparation of materials like wound dressing, antiviral and antifungal agents, dialysis membranes Biomedical beads, Fabrics and gauzes . Chitosan is a wound healing accelerator, and its effectiveness in protecting wound from bacterial invasion by suppressing bacterial proliferation. It may cat as effectively against typhoid producing microorganism . The result of present study one kg of prawn Macrobrachium rosenbergii sample produced 580gms of biowaste and Penaeus monodon produced 455gms of biowaste. In case of yield goes to 124.8 gms and
109.2 gms in the sample.

It has been observed that the percentage of chitosan yield from shrimp waste collected from Penaeus

Table.1 Yield of bio-waste from two experimental organisms

Experimental Species	Total sample weight (g)	Wet Bio-waste (g)	Dry weight of crude (g)	Chitosan yield per Kg of sample (g)
Macrobrachium rosenbergii	1000	580	124.8	32.44
Penaeus monodon	1000	455	109.2	26.64

Table.2 Proximate analysis of exoskeleton *Macrobrachium rosenbergii* and *Penaeus monodon*

Parameter	*Macrobrachium rosenbergii*	*Penaeus monodon*
Moisture (%)	75.2 ± 5.6	76.2 ± 5.7
Ash Content (%)	33.25 ± 3.38	34.9 ± 3.87
Proteins (%)	31.25 ± 2.38	28.89 ± 2.17
Fats (%)	4.77 ± 0.15	6.77±0.95

semisulcatus is found to be 32.25% , chitosan yield from biowaste of Penaeus carinatus and Penaeus monodon was found to be 34% ,it is reported as 18.6% , 30% , 17% , chitosan yield from biowaste of Penaeus monodon, chitosan yield from the biowaste of Penaeus monodon was found to be 67.47% and 46%

It is obvious that the amount of chitosan yield is proportional to the amount chitin obtained from the bio-waste of shellfish, the amount of chitin yield intern depend on the amount of biowaste obtained from shellfish. Partially acetylated chitosan polymers exhibit a number of biological activities, including antimicrobial activities, elicitor activities inducing disease resistance in plants, and diverse stimulating or inhibiting activities towards a number of normal or transformed human cell types. Purified and well characterized chitosan showed biological activities correlated with physico-chemical properties of the polymers used. The source made from waste (chitosan) shows an excellent antimicrobial activity against human pathogens. Thus, it can be used as good potent source against the infectious pathogens.

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