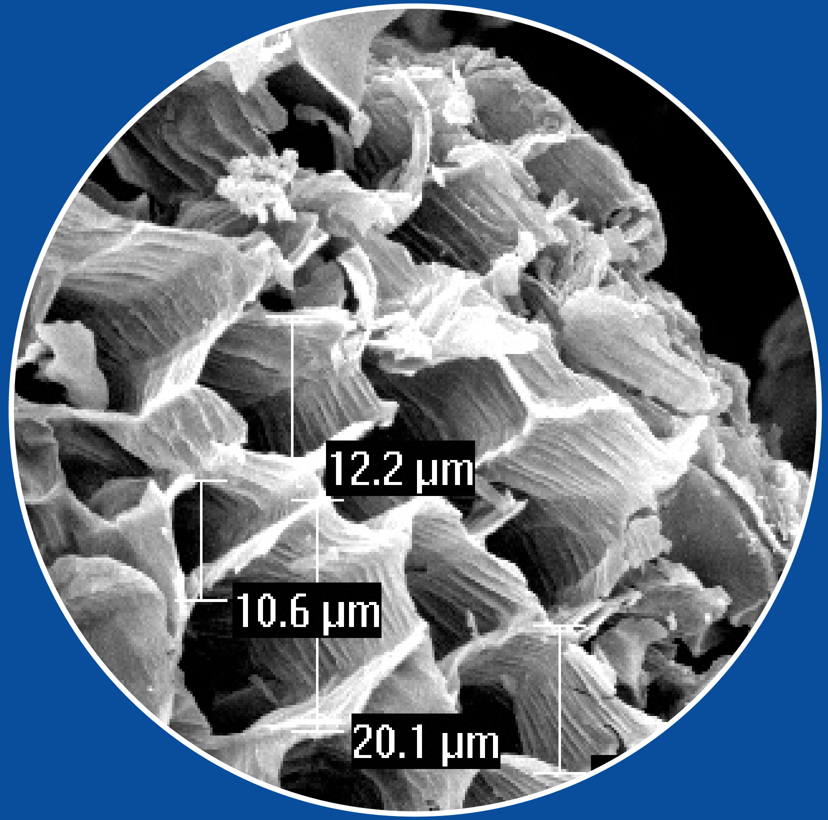
Training Manual

Skill Development Programme
on

Biochar for Enhancing the Aquaculture Productivity

3-7 March, 2020



Sponsored by
ICAR - National Agricultural Higher Education Project

ICAR-Central Institute of Fisheries Education
(Deemed University)
Mumbai
www.cife.edu.in

Manual on Skill Development Programme

on

Biochar for Enhancing the Aquaculture Productivity

3-7 March, 2020

Under the aegis of

National Agricultural Higher Education project (NAHEP)

Compiled and Edited by

Vidya Shree Bharti
Gayatri Tripathi
Saurav Kumar

ICAR-Central Institute of Fisheries Education
(University under Sec.3 of UGC Act)
Panch Marg, Off Yari Road, Versova
Mumbai-400061
Website: www.cife.edu.in

Training Manual

Skill Development Programme on “Biochar for Enhancing the Aquaculture Productivity”

3-7 March, 2019

Programme Director
Dr. Gopal Krishna

Course Director
Dr. K.V. Rajendran

Course Coordinators
Dr. Vidya Shree Bharti
Dr. Gayatri Tripathi
Dr. Saurav Kumar

Cover Design
Dasari Bhoomaiah

Assisted by
Mr. Ashutosh Kumar Singh
Mrs. Iffat Jahan
Mr. Vijay Kumar A

Printed and Published by
Dr. Gopal Krishna
Director & Vice-Chancellor
ICAR-Central Institute of Fisheries Education
(University under Sec.3 of UGC Act)
Panch Marg, Off Yari Road,Versova, Mumbai-400061
Phone: 022-26361446/7/8 Fax: 022-26361573
www.cife.edu.in

भा.कृ.अ.प. - केन्द्रीय मात्स्यिकी शिक्षा संस्थान
ICAR-CENTRAL INSTITUTE OF FISHERIES EDUCATION
(A university Established Under Sec.3 of UGC Act 1956)
Ministry of Agriculture, Govt. of India

डॉ. गोपाल कृष्णा
निदेशक एवं कुलपति
Dr. Gopal Krishna
Director & Vice-Chancellor

FOREWORD

The ICAR-Central Institute of Fisheries Education is playing a pivotal role in Human Resource Development in major disciplines of Fisheries Science. Apart from conducting post-graduate programs, the faculty members are also involved in imparting trainings in the emerging areas of fisheries and aquaculture. The management of Agro-waste through biochar production and its application in the system is one such area which has got tremendous potential for improving the soil and water quality to enhance aquaculture productivity, carbon sequestration, removal of contaminants and greenhouse gas mitigation. ICAR-CIFE has taken up this emerging research area and has established the working facilities for the research and trainings in the Aquatic Environment and Health Management Division.

The Skill Development Programme (SDP) on "Biochar for Enhancing Aquaculture Productivity" sponsored by NAHEP is a well-designed programme and includes designing of Biochar klin, its production, characterization and application for water and sediment management. I am sure that the course manual prepared under this programme would serve as a useful reference material for the students, farmers and entrepreneurs working in the relevant field of aquaculture and fisheries sector. I congratulate Dr. K. V. Rajendran, Head of the Division and Course Director and Dr. Vidya Shree Bharti, Dr. Gayatri Tripathi and Dr. Saurav Kumar, the coordinators of the program and all the faculty members of Aquatic Environment and Health Management Division for successfully organizing this training program.

Place: Mumbai
Dated: 3rd March, 2020

(Gopal Krishna)

पंच मार्ग, ऑफ यारी रोड, वरसोवा, अंधेरी (प.), मुंबई - ४०० ०६१. (भारत)
Panch Marg, Off Yari Road, Versova, Andheri (W), Mumbai - 400 061. (India)
कार्यालय / Office) : 022-26361446/7/8,
Fax : 022-26361573
Website : http ://cife.edu.in

भा.कृ.अ.प. - केन्द्रीय मात्स्यिकी शिक्षा संस्थान
ICAR-CENTRAL INSTITUTE OF FISHERIES EDUCATION
(A university Established Under Sec.3 of UGC Act 1956)
Ministry of Agriculture, Govt. of India

PREFACE

The research in the field of biochar is advancing at a rapid pace leading to new and varied applications. Biochar with desired characteristics is now used by researchers working in the field of agricultural and aquaculture sectors. The Skill Development Programme (SDP) on "Biochar for Enhancing Aquaculture Productivity" sponsored by National Agricultural Higher Education Project (NAHEP) is conceptualised for farmers, entrepreneurs and students to gain hands-on training on biochar preparation and its applications. The programme has been designed to provide theoretical and practical exposure to participants on production, characterization and applications of biochar technology.

A total of thirteen participants have been trained under this programme. The training included lectures, practical sessions and a field visit to ICAR-CIRCOT, Matunga, Mumbai. The resource persons engaged in this training were from different disciplines to acquaint the participants with the applications of designer biochar in the aquatic system. The lecture notes and practical methods contributed by the resource persons have been compiled in this manual. The training manual would be a useful reference material for the participants as well as for the students.

We would like to place on record our sincere gratitude to Dr. Gopal Krishna, Director & Vice-chancellor, ICAR-CIFE for his unstinted support and guidance in organizing this training programme. We are extremely obliged to ICAR-World Bank sponsored NAHEP for funding this training programme. The Course Director and Coordinators would also like to acknowledge the contributions of all the resource personnel and support from scientists, staff and students of the AEHM Division.

K.V. Rajendran	Gayatri Tripathi	Vidya Shree Bharti	Saurav Kumar
(Course Director)	(Course Coordinator)	(Course Coordinator)	(Course Coordinator)

CONTENTS

1. Importance of Biochar in Aquaculture

Vidya Shree Bharti & Chittaranjan Raul
ICAR- Central Institute of Fisheries Education, Versova, Mumbai-400061

Introduction

Aquaculture has emerged as a key food production sector representing about 25% of protein source. It plays an important role in generating income and employment opportunities for a large population of the world. Global fish production stands at 167.1 million tonnes, of which about 42% is contributed by aquaculture sector. With an average growth rate of 8.3% per year, it is one of the fastest growing industries in the world. The intensive aquaculture practices, encouraged by the quest for higher revenue, causes environmental problems like coastal deforestation, salinization, eutrophication, groundwater contamination, damage of agriculture land, etc. Use of extrinsic chemical compounds further manifests these problems, causing a threat to the environmental sustainability of the system. These chemical compounds can be suitably replaced by alternative organic materials, which can enhance the sustainability of the system without undermining its profit generating capacity. Biochar, which has been extensively used in agriculture for productivity enhancement, can also be used for aquaculture for similar purposes. However, there are fewer evidences of practice and research on the use of biochar in aquaculture. Biochar is a fine-grained, carbon-rich, porous product, produced from agricultural, industrial, urban, and animal waste, by pyrolysis at a temperature 350-600°C in absence of oxygen. The type of biomass and temperature are the deciding factors for physicochemical properties and nutrient content of biochar. It has a higher potential for use in aquaculture by virtue of its novel physicochemical properties such as the high surface area to volume ratio, functional groups, trace elements, absorption property among others.

Biochar Application in Aquaculture

In aquaculture, biochar can be a vital input, with its potential uses ranging from the pH adjustment of soil and water to the reduction in the greenhouse gases from the system. Some of the identified potential uses of the biochar have been discussed below.

Acid Neutralisation

The pH of biochar remains in the alkaline range and contains calcium, magnesium and carbonate ion (identified by FT-IR analysis). Use of biochar can reduce the lime application in aquaculture ponds, by acting as an alternative, owing to its good buffering capacity (Schmidt and Wilson 2012).

Reduction in Seepage and Leaching Loss of Nutrients

Biochar application in the pond bottom can reduce the seepage by enhancing the Water Holding Capacity (WHC). Further, the Anion Exchange Capacity (AEC) of biochar can retain the nitrate ions produced from the feed and fecal waste decomposition. These can particularly be useful in inland saline aquaculture ponds of Haryana, Punjab, and Rajasthan, which are facing high seepage loss, due to the very less organic carbon in sediment causes withdrawal of ground saline water for aquaculture. Leaching loss of nutrient particularly nitrate will lead to contamination of groundwater and will be the major future problem for safe drinking water

Enrichment of Mineral Nutrients

The mineral nutrient concentration of inland saline pond water is not same as brackish or sea water. It results in muscle deformities of shrimps due to deficiency of potassium (minimum 50% potassium level of seawater required for) and imbalance in Ca: Mg ratio (minimum 1:2.25 required in inland saline water for shrimp). The biochars produce from banana peduncle; sugarcane bagasse and other agro-waste are rich in potassium, calcium and magnesium which can supplement these minerals (Dahlawi et al. 2018).

Use as a Feed Additive

Research shows the incorporation of biochar as a feed additive increases the growth rate in catfishes (Lan et al., 2016). It improves ammonia absorption from the fecal matter and hence reduces the ammonia toxicity in high-density larval rearing units.

Use as Plant Bed in Aquaponics System

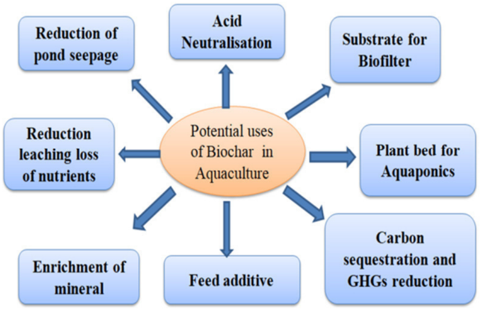
Biochar beads are used as plant substrate in aquaponics system. It retains nutrient from wastewater, and facilitates symbiotic association of mycorrhizal fungus with plant roots, which helps in mineral and nutrient supplement to plant from the water.

Substrate for Biofilter

The biochar removes pesticide and heavy metals by adsorption mechanism, making it more suitable for use as a substrate in biofilter and Effluent Treatment Systems (ETS) of shrimp farm. The diversified microbial association property, and bio film formation capacity of biochar, makes it more suitable for the above purposes (Nguyen 2015).

Carbon sequestration

Biochar helps in global warming mitigation by carbon sequestration and reducing the greenhouse gas emission from the agriculture fields. Research evidences put the capacity of biochar to sequester to a level of 12% of anthropogenic CO_2 equivalent gas per year. Use of biochar in aquaculture can reduce the emission from the system, and hence contributing to the mitigation. However, more researches are called for to bring out other potential uses of biochar, which has largely remained unexplored.



(Use of biochar in aquaculture)

(Agro-waste)

(Biochar produced using electrical kiln)

(Bagasse biochar)

Conclusion

Agro-waste is usually considered as non-useable resources. India produces about 550 thousand tons of agro-waste per year which can be successfully converted to biochar for deriving maximum benefit with "waste to wealth" concept. Although the potential effect of biochar application in agriculture field is well studied, application in the aquaculture is still unexplored. Furthermore, the differential characteristics of biochar from different agro-waste needs to be scientifically studied for identifying the best potential use. While ensuring several benefits for farming systems, biochar simultaneously ensures less environmental footprints by reducing greenhouse gas emission. Hence, the application of biochar in aquaculture field is a futuristic approach for both better productivity as well as sustainability.

References

- Dahlawi, S., Naeem, A., Rengel, Z. and R. Naidu. 2018. Biochar application for the remediation of salt-affected soils: Challenges and opportunities. Science of the Total Environment 625:320-335.
- Lan, T. T., Preston, T. R. and R.A. Leng. 2016. Feeding biochar or charcoal increased the growth rate of striped catfish (Pangasius hypophthalmus) and improved water quality. Livestock Research for Rural Development 28:36-46.
- Nguyen, T. 2015. Adsorption Of Phosphorus From Wastewater Onto Biochar: Batch and Fixed-bed Column Studies.
- Schmidt, H.P. and K. Wilson. 2012. 55 uses of biochar. Itahaka-Journal 1:286-289.

2. Production and physical Characterisation of Biochar

Vidya Shree Bharti, Ashutosh Kumar Singh & Vijay Kumar A
ICAR- Central Institute of Fisheries Education, Versova, Mumbai-400061

Biochar (BCs)

BCs are made from range of biomasses that have different chemical and physical properties. The properties of each biomass feedstock are important in thermal conversion processes, particularly the proximate analysis (ash and moisture content), caloric value, fractions of fixed carbon, and volatile components, percentage of lignin, cellulose, and hemicelluloses, percentage and composition of inorganic substance, bulk, true density, particle size, and moisture content. Extensive feedstock biomasses have been used in the production of BC such as bioenergy crops (willows, miscanthus, and switchgrass), forest residues (sawdust, grain crops, and nut shells), organic waste (green yard waste and animal manure), agricultural waste, kitchen waste, and sewage sludge. It was again found that the following temperature-dependent structure transition was proposed: (1) transition BC having crystalline nature of the biomass feedstock or material preserved; (2) amorphous BC that is a random mixture of thermally changed molecules and emerging aromatic polycondensates; (3) composite BCs having poorly structured graphene stacks fixed in their amorphous phase; and (4) turbastic BC dominated by chaotic graphite crystallites. In general, a high yield of BC derived from this biomass which has more lignin and less cellulose can be expected. Meanwhile, the porosity of BC increases with the content of lignin in biomass. In addition, the volatile component, water content, and particle size and shape of biomass will affect the property of BC obtained. BCs formed at higher temperatures (lower oxygen to carbon ratio) are expected to be -donors, while BCs formed at lower temperatures are expected to be π-acceptors. BC produced from agricultural waste biomasses does not cause any notable life cycle based greenhouse gas (GHG) emissions.

Preparation of BC

Carbonised organic matter can essentially have different physical and chemical properties based on the technology (e.g., torrefaction (a pyrolytic process primarily at low temperature), slow pyrolysis, intermediate pyrolysis, fast pyrolysis, gasification, hydrothermal carbonization (HTC), or flash carbonization) used for its production. In contrast to considerable research, this has already been carried out to assess the value of BC as soil amendment. No publication was identified which looks at the use of BCs from modern pyrolysis as soil amendment and immobilization of both inorganic and organic contaminants in the soil and water systems. BC can be produced both in traditional earthen charcoal kilns, where pyrolysis, gasification, and combustion process are carried out in parallel below the earthen kiln layer, and in modern BC retorts, where pyrolysis and combustion processes are physically separated by a metal barrier.

Pyrolysis technology can be distinguished by the residence time, pyrolytic temperature of the pyrolysis material (e.g., slow and fast pyrolysis process), pressure, size of adsorbent, and the heating rate and method (e.g., pyrolysis started by the burning of fuels, by electrical heating, or by microwaves).

Slow pyrolysis (heating for seconds or minutes) may be described as a continuous process, where purged (oxygen-free) feedstock biomass is transferred into an external heated kiln or furnace (gas flow removing volatile BC emerging at the other end); “fast” pyrolysis on the other hand depends on very quick heat transfer, typically to fine biomass particles at less than 650°C with rapid heating rate (ca 100–1000°C/s). The characteristics of the BC product are heavily affected by the extent of pyrolysis (pyrolytic temperature and residence pressure) and entirely by biomass size and kiln or furnace residence time. The rate at which volatile and gases are removed in a kiln or furnace determines the vapour residence time. Prolonged residence time results in secondary reactions, notably the reactions of tar on BC surfaces and charring of the tar rather than additional combustion or processing outside the kiln or furnace. For gasification in pyrolysis, the biomass feedstock to some extent is oxidized in the gasification chamber at a temperature of about 800°C at atmospheric or elevated pressure. As already pointed out by its name, the main product of this process is gas; only few or no BCs, liquids, or the likes are formed.

Physical Charectrisation of Biochar

Bulk Density

Principle

The mass of the biochar is determined by weighing the oven dry soil sample. The in small amounts, say 5-6 gm., is placed in a container which is tapped 15-20 times on a table be letting it fall from a height of about 2-3 cm. This tapping is assumed to produce the same packing as occurring naturally in the field, even though this assumption is not strictly correct. The volume of this packed biochar will be equal to the volume of the container. Bulk density is calculated from the mass and volume of the biochar.

Purpose

The bulk density varies indirectly with total pore space present in the soil and gives a good estimate of porosity of soil. Bulk density is of great importance than particle density in understanding the physical condition of soil. Soil bulk density is defined as the ratio of the mass of the oven dry soil to its bulk volume.

Different biochar bulk density varies from 0.08 g cm^{-3} to 0.43 g cm^{-3} depending on feedstock biomass and process conditions, is lower than that of mineral soil ranging from 1.16 to 2.00 g cm^{-3}. The applied bagasse biochar bulk density is 0.24 g cm^{-3} which is very less than the saline sediment 0.09 g cm^{-3}.

Apparatus

A weighing bottle (50 ml), balance and a burette of 50 ml capacity.

Procedure

Weigh an empty 50 ml bottle. Fill the bottle with oven dry soil up to the brim by tapping and weigh it. Empty the bottle and determine its exact volume using burette.

Calculation

Mass of empty bottle = M1 gm.

Mass of bottle = M2 gm.

Mass of the biochar = (M2 – M1) gm.

Volume of water filling the bottle = V cm^3.

Bulk density = (M2 – M1) / V $gm.cm^{-3}$ or $mg.\ m^{-3}$

Water Holding Capacity

Principle

The macro and micro pores present in the particle or any matter holds the moisture or water by capillary action.

Purpose

The information about WHC of biochar helps in knowing seepage rate and the organic matter status of pond sediment.

Procedure

- Crush biochar sample and pass through 2 mm sieve.
- Place round filter paper and fix it to the internal perforated floor of the aluminium moisture can.
- The weight of the dish and filter paper is noted.
- The dish is then filled with soil by tapping the dish briskly & making plane the top of biochar and find out its weight.
- Place the set of perforated dishes in enamel tray.
- Pour the water in enamel tray at half of height of dish.
- Water may rise in dish through perforated bottom and moist the biochar to its capacity.
- Keep it for 5 to 6 hours in water. Take the dishes and place it on a filter paper sheet, so that the excess of water may drain away from the pores within half an hour.
- The dish containing moist biochar is weighted and the weight is noted.

Observations

1) Weight of empty dish + filter paper - a gms.

2) Weight of empty dish + filter paper - b gms. + air dry soil

3) Weight of empty dish + filter paper - c gms. + wet soil

Calculations

Saturation Moisture % = $(c-a)-(b-a)/(b-a)$x 100

Ash

The mineral portion remaining after complete burning of biochar is called ash.

Requirements

Crucible

Muffle furnace

Biochar

Procedure

- Take biochar of known weight
- Weight of crucible also taken before keeping with biochar.
- The total weight of biochar and crucible taken.
- Keep the crucible in muffle furnace at 760 ^{o}C for 6 hr.
- Measure the total weight of crucible after cooling it.

Calculation

Ash contain of biochar= W1-W2

W1= Crucible+ biochar wt initial

W2= Crucible +biochar wt final

3. Chemical Characterisation of Biochar

Vidya Shree Bharti & Vijay Kumar A
ICAR- Central Institute of Fisheries Education, Versova, Mumbai-400061

pH

Principle

The electrometric determination of pH by a pH meter is based on measuring the e.m.f. (milivolts) of a pH cell both a reference buffer then with a test solution. The change in the potential difference at 25°C for 1 pH unit is 59.1 mV. The pH of a biochar is a measure of the hydrogen or hydroxyl ion activity of the soil – water system. It indicated whether the soil is acidic, neutral or alkaline in reaction.

Purpose

The determination of pH in soil is important as it plays a great role in availability of nutrients to plants. This determination can be done more accurately in the laboratory by electrometric method. pH determination is useful for soil classification on the basis of acidity or alkalinity.

Reagents

Standard Buffer Solutions

Dissolve one commercially available buffer tablet each of pH 4.0, 7.0 and 9.2 in freshly prepared distilled water separately and make up the volume to 100 ml. Prepare the fresh solution every week as these solutions are unstable. Alternatively the buffer solutions can be prepared in the laboratory as given below.

Apparatus

i) pH meter with glass electrodes
ii) Thermometer
iii) Glass beaker (100 ml)
iv) Glass rod

Procedure

- Weigh 2.5 gm of 2.0 mm air dry biochar into a beaker. Add 50 ml of distilled water and stir with a glass rod thoroughly for about 5 minutes and keep for half an hour.
- In the meantime turn the pH meter on, allow it to warm up for 15 minutes. Standardize the glass electrode using standard buffer of pH = 7 and calibrate with the buffer pH = 4 or pH = 9.2.
- Dip the electrodes in the beakers containing the soil water suspension with constant stirring.
- While recording pH, switch the pH meter to pH reading, wait 30 seconds and record the pH value to the nearest 0.1 unit.

EC (Electrical Conductivity)

Principle

The electrical conductivity of water extract of biochar gives a measure of soluble salt content of the soil. Pure water is very poor conductor of electric current, whereas water containing the dissolved salts in biochar conducts current approximately in proportion to the amount of soluble salts present. Based on this fact, the measurement of electrical conductivity of an extract gives a satisfactory indication of the total concentration of ionized constituents.

Purpose

Salted soils are classified on the basis of two criteria, one is on the basis of total soluble salt (TSS) content and another is exchangeable sodium percentage (ESP) or more recently sodium Absorption ration (SAR). Ions in water conduct electrical current, therefore electrical conductivity is fast, simple method of estimating amount of total soluble salt (TSS) in soil sample. Electrical conductivity is expressed in dS/m.

Apparatus

1) Digital conductivity meter

2) Conductivity cell.

3) Glass beaker (100 ml)

4) Glass rod

Reagent

0.01N Potassium chloride solution: Dry a small quantity of A.R. grade Potassium chloride at 60°C for 2 hours. Weigh 0.7456 gm of it and dissolve in freshly prepared distilled water and make to one litre. This solution gives and electrical conductivity of 1411.8 x 10^{-3} i.e. 1.41 dS/m at 25°C.

Procedure

- Calibrate the conductivity cell with the help of standard KCL solution and determine the cell constant.
- The soil water suspension of 20 gm : 50 ml ratio prepared for the determination of pH can also be used for conductivity measurements. After recording the pH, allow the soil water suspension in the beaker to settle for additional half an hour (the total intermittently shaking period is 1 hr.)
- After the calibration dip the conductivity cell in the supernatant liquid of the soil water suspension. Read the conductivity of test solution in proper conductance range.
- Remove the cell from soil suspension, clean with distilled water and dip into a beaker of distilled water. EC is expressed as $dS.m^{-1}$.

Cation Exchange Capacity of Biochar

Principle

The cation exchange capacity (CEC) determination involves measuring of total quantity of negative charges per unit weight of the biochar which are neutralize by the exchangeable cations. It is defined as the capacity of the biochar to adsorb the sum total of exchangeable cations expressed as milliequivalents per 100.0 gm of soil or C mol (P^+)/kg of biochar on oven dry basis. Clay particles and organic matter carry negative charges over their surface due to which they adsorb positively charged particles (cations). There are different types of cations. e.g.: H^+, Ca^{++}, K^+, $NH_4{}^+$, Mg^{++} etc.

These cations can replace each other depending upon their concentration (mass action) andreplacing power. The replacement of cations by one another is known as cation exchange. The order of replacing power of some cations are given below:
$H^+ > Ca^{++} . Mg^{++} > K^+ > NH_4^+ > Na^+ > Si^{++++}$

Reagents

- Ammonium Acetate, 1N, pH 7.0 + 0.1: Dissolve 77.08 gm of ammonium acetate in distilled water and makeup the volume to one litre. Adjust the pH at 7 with ammonia or acetic acid.
- Potassium Chloride (10%, pH 2.5 + 0.1): Dissolve 10 gm of potassium chloride in distilled water and make up the volume to 100 ml. Adjust the pH at 2.5 with 1N HCl.
- Methanol or ethanol, 60%: Dilute 60 ml methanol with distilled water and make up the volume to 100 ml.
- Sodium hydroxide, 40%: Dissolve 400 gm NaOH in water in a 1 litre volumetric flask. Let it cooled and make it upto the mark.
- Methyl red: Dissolve 0.1 gm methyl red in 100 ml ethanol (98%).
- Hydrochloric acid (0.02N): Take 1.8 ml of concentrated HCl in one litre volumetric flask and make the volume with distilled water.

Procedure

- Weigh 10.0 gm of 5 mm sieved air dried soil sample into 500 ml plastic conical flask. Add 250 ml of neutral ammonium acetate solution.
- Shake the contents on mechanical shaker at 110 rpm for an hour and keep it over night. Next day again shake it for 1 hour.
- Filter the contents through Whatman No. 1 filter paper receiving the filtrate in a 250 ml volumetric flask.
- Transfer the soil completely on the filter paper and continue to leach the soil with the neutral solution (using 20-25 ml at atime).
- Allowing the leachate to drain completely before fresh aliquot is added. If it is not enough add some ammonium acetate to make up the mark. The solution is now ready for determination of individual cations.
- The residue left on the filter paper is intended for the determination of cation exchange capacity of soil. Wash the leached soil with 60% methanol or ethanol to remove the excess ammonia. Each time 10 ml of alcohol in interval is added, draining between each addition is necessary. Nessler's reagent does the test of ammonia. If Nessler's reagent is not available the test of ammonia is done by HCl.

- Collect the leachable washing of ethanol / methanol in watch glass and add few drops of concentrated HCl if it fumes that indicates presence of ammonia otherwise not.
- After washing, the soil is leached with acidified potassium chloride (10%, pH= 2.5+0.1) and the extract is collected finally in a 250 ml volumetric flask.
- 25–30 ml of KCl solution is added. Each time draining between each addition is necessary. The volume is adjusted to the mark by the replacing solution of KCl. It is shaken to make the concentration solution homogeneous.
- Transfer 20 ml of KCl leachate and 10 ml of distilled water in a Gerhardt tube, add 10 ml of 40% NaOH and distilled into 20 ml of HCl (0.02N) containing 6 drops of methyl red indicator. The approximate distillate is collected about 150 – 200 ml. Titrate the solution until the yellow colour appears with 0.02N NaOH.
- The changes from red to yellow, the end point being taken at the first appearance of the yellow colour. 20 ml of 10% KCl solution is distilled in the same manner for blank.

Calculations

- D = The weight of oven dry soil in the weight of air dry soil taken for analysis.
- V = The total volume of the final solution containing the ammonium ion.
- T = The volume of standard acid (0.02N) used for titrating the ammonia nitrogen after correction for the blank.
- N = Normality of standard acid 0.02N
- If 20 ml of ammonium solution is distilled
- If 20 ml of ammonium solution is distilled
- ❖ Cation exchange capacity = NTV*100 / 10 D (meq/100 gm of soil.)

Available Nitrogen Content

Apparatus:

Kjeldahl Digestion Assembly, Ammonia Distillation Assembly.

Principle:

The Kjeldahl method permits the available nitrogen to be precisely determined in the plant and in the soil. The method of determination involves three successive phases which are-:

- Digestion of the organic material to convert nitrogen into HNO3.
- Distillation of the released Ammonia into an absorbing surface or medium.
- Volumetric analysis of the Ammonia formed during the digestion process.

Digestion

Digestion of the organic material is carried out by digesting the sample with Con. H_2SO_4 in the presence of $CuSO_4.H_2O$ as a catalyst and K_2SO_4 which raise the digestion temperature.

Distillation:

The Ammonia content of the digest is determined by distillation with excess NaOH and absorption of the evolved NH_3 is in standard HCl.

Volumetric Analysis:

The excess of standard HCl is titrated against standard NaOH using Methyl Red as an indicator. The decrease in the multi equivalence of acid as determined by acid-base titration, which gives a measure of the N content of the sample. The end point is determined by a change of colour from pink to yellow.

4. Biochar for Primary Productivity

Vidya Shree Bharti & Vinod Kumar Yadav
ICAR- Central Institute of Fisheries Education, Versova, Mumbai-400061

Biochar is a pyrolyzed material produced from different organic and agro-waste. Nowadays it has wide spectrum uses like livestock feeding, filtration, wastewater treatment, gardening, packaging material, textile industry, 3D printing, fuel cell, carbon sequestration, and many others. Agriculture focuses on major consumption areas for biochar uses. It acts as a soil conditioner that improves the productivity of crop production by multifunctional abilities like improving physic-chemical and biological properties of soil.

- ✓ Increases crop production per unit area.
- ✓ Reduce fertilizer quantity for crops.
- ✓ Increases root surface area for nutrient absorption by providing symbiotic of actinomycetes with plant roots.
- ✓ Climate change mitigation by reducing carbon dioxide and methane emission.
- ✓ Harvest diverse microbes that increase microbial nutrient cycling.
- ✓ Improve soil Physico-chemical properties

Effects of Biochar on Plant Growth

With the modification to soil characteristics described above, the effect of biochar additions to soil on plant productivity is the most important outcome for its use in Australian agriculture. Evidence gathered from both glasshouse and field trials indicates that biochar additions to acidic and nutrient-poor soils, combined with fertilizer application, can produce yields greater than either fertilizer or biochar alone. However, the effect of biochar on crop growth depends on application rates and the soil type to which it is applied. A key feature of biochar addition to soils is increased nitrogen use efficiency by plants. The evidence suggests that significant reductions in nitrogen fertilizer application can be achieved while maintaining similar yields, with the addition of biochar to soils. Alternatively, yields may increase significantly with the addition of biochar to soils and little change in established nitrogen fertilizer regimes.

Case studies of biochar improve agriculture productivity

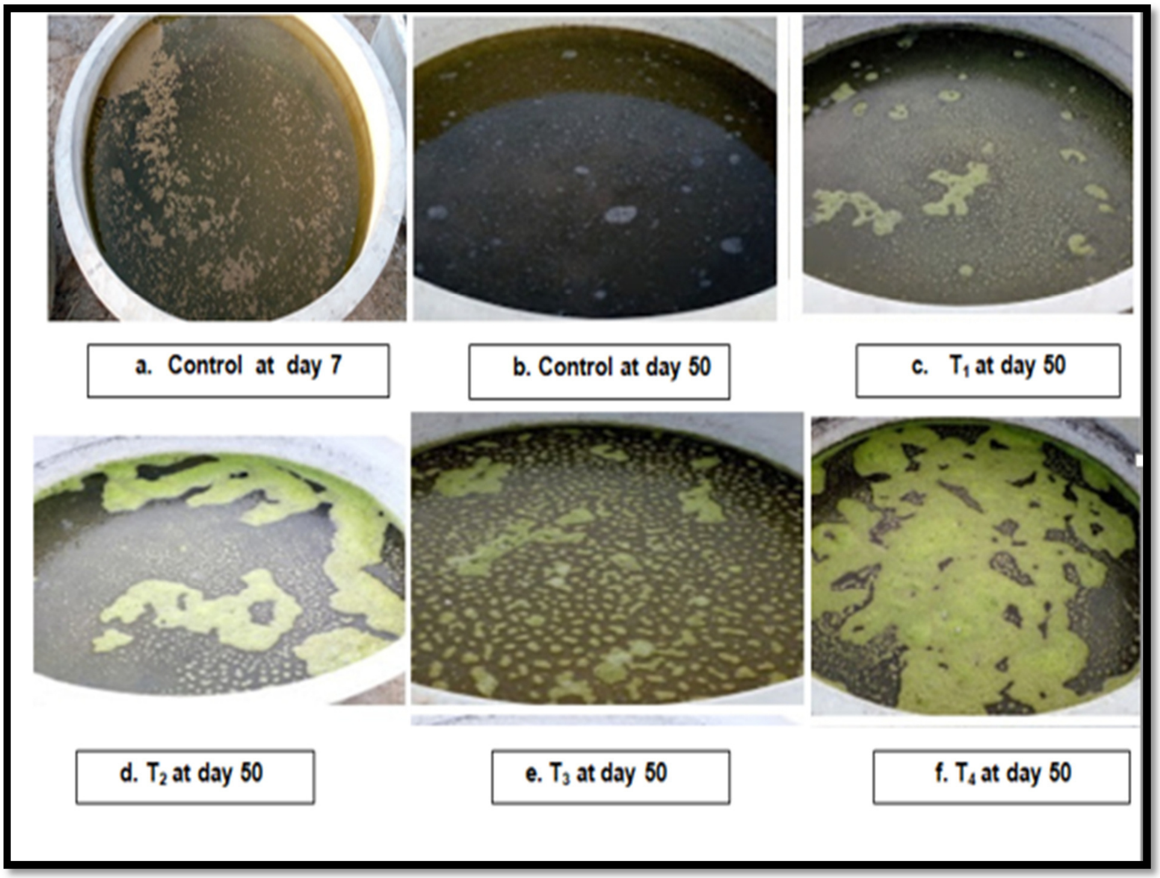
- ✓ Qin et al., (2016) conducted a 4-year study on the effect of biochar application on rice yield in south China. Wheat straw Biochar applied at a rate of 5 tha-1 (BC 1), 10 t ha^{-1} (BC 2) and 20 t/ha (BC3) with base chemical fertilizer (N: P_2O_5: K_2O= 15%:15%:15%), for each treatment was broadcast manually. There was an increase in rice yield by 2.82, 3.56, 7.47% in BC1, BC2 treatments BC3 respectively.
- ✓ Zhang et al., (2010) performed a field trial to investigate the effect of biochar application on rice yield with or without N fertilizer from Tai Lake plain, China. Biochar of wheat straw (pyrolyzed at 350-450 °C) amendments at the rate 10 t ha^{-1} and 40 t ha^{-1} increased rice yields by 12% and 14% in unfertilized soil, and by 8.8% and 12.1% in soils with N fertilizer, respectively.
- ✓ Uzoma et al., (2011) investigate the effect of cow manure biochar on maize yield and physicochemical properties of dry land on sandy soil. Biochar was derived from dry cow manure pyrolyzed at 500° C. Cow manure biochar was mixed with sandy soil at the rate equivalent to 0, 10, 15 and 20 t ha^{-1}. Maize yield and nutrient uptake were significantly improved with increasing the biochar mixing rates. Application of biochar at 15 and 20 t ha^{-1} significantly increased maize grain yield by 150 and 98% as compared with the control, respectively. Results of the soil analysis after the harvesting indicated a significant increase in the pH, total C, total N, Olsen-P, exchangeable cations and cation exchange capacity.
- ✓ Chan et al., (2008) made a pilot study on yield assessment of radish (Raphanus sativus) yield by application of poultry biochar. Two types of biochar produced from poultry litter at 450 °C and 550 °C. The biochar applied at the rate of,10, 25,50 t ha^{-1} with (180 kg N/ha) and without nitrogen fertilizer to the radish field. This study shows that there was a similar dry yield of radish in biochar with or without N fertilizer treatment which is detectable at the lowest biochar application rate (10 t ha^{-1}). The yield % compared with control rose to 42% t ha^{-1} in 10 and 96% in 50 t ha^{-1} in the biochar amendment. In this regard, the biochar produced at 450°C is more effective than 500 °C due to the presence of more available phosphorous nutrient at low pyrolyzed temperature.
- ✓ Jones et al., (2012) investigated the multilayer effects of biochar on crop productivity and soil quality with specific emphasis on carbon, nitrogen cycling in 3 year period.

Biochar was added to the agriculture field at 0, 25, 50 t ha^{-1} and planted with maize (1st year) and grass (2nd and 3rd year). The result shows that there was a significant increase in crop production by 24% and increased foliar nitrogen (2nd year) and above-ground biomass. Below ground, biochar increases soil respiration, fungal and bacterial population in 2nd year. Biochar did not affect the Dissolved organic carbon (DOC), Dissolved organic Nitrogen (NH^{4+}, NO^{3-}). The alkalinity associated with biochar has been fully neutralized and lost most of its citations (K, Ca, Mg) at the end of the 3rd year.

- ✓ Schulz et al., (2013) studied the optimum biochar and compost ratio on plant productivity and soil fertility. Biochar applied at 0 to 50 t ha^{-1} in 18 combinations with compost to Oat plants (*Avena sativa*) in a fully randomized greenhouse study with sandy loam soil bed. Results show that plant biomass production was increased by 30% with rising biochar and compost amounts. Oat plant height and seed weight was improved only with rising biochar amounts, but not with compost amounts.

Biochar Increases Primary Productivity in the Aquaculture System.

The primary productivity of pond water means the presence of autotrophs. Its availability also influences by the nutrient status of the water body. The chlorophyll-a concentration of inland saline pond water is very poor (0.088 to 0.0913 mg m^{-3}). The biochar added into the water surface and sediment mixing before water addition @ 9 t/ha and 18 t/ha. The instability in water parameters (alkalinity, pH, TSS) and a smaller number of phytoplankton's planktons may delay the reappearance of primary productivity. After a month addition of biochar (produced from sugarcane bagasse), there was an increase in basic nutrients in the water column, a decrease in turbidity and sunlight availability causes an increase of primary production. The significant increase in the nutrient level of water due to the microbial supplement (mainly N nutrient) from sediment and available nutrient release from biochar application. As biomass of primary producer increases, it causes a decrease in NH_4^+, NO_3^-, PO_4^{3-} a nutrient in the water. It is reported that the biochar amendment increase plant (maize, radish, acacia) growth in the agriculture field of normal and saline soil. Aquatic ecosystem productivity is different from the agriculture ecosystem but from above water quality parameter observations, we can conclude that the nutrient availability from biochar to the water column causes a significant bloom of primary productivity.



a. Control at day 7

b. Control at day 50

c. T_1 at day 50

d. T_2 at day 50

e. T_3 at day 50

f. T_4 at day 50

5. Biochar Application in Wastewater Treatment

Vidya Shree Bharti, Vijay Kumar A & Amal C.T
ICAR- Central Institute of Fisheries Education, Versova, Mumbai-400061

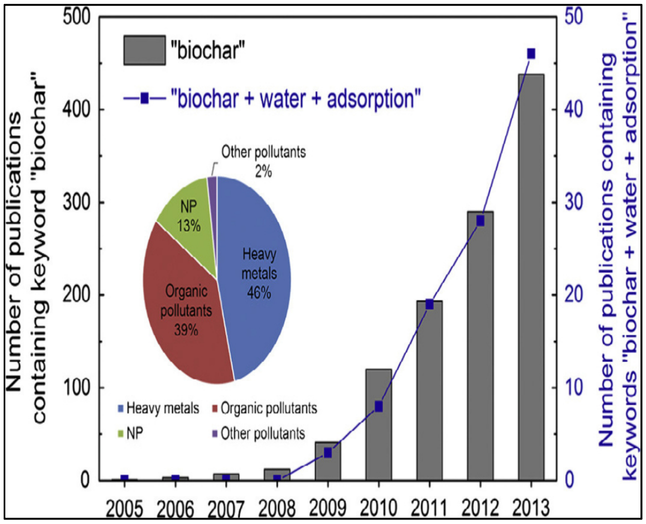
The pollution of water bodies by organic, inorganic and trace elements has continued to attract significant global attention from government regulatory agencies and the general public. Many organic compounds are persistent or can be biodegraded or transformed into recalcitrant products that survive conventional water treatment processes. In recent years water treatment and purification have drawn much attention due to the efficient utilization of waste and contaminated water in a sustainable way. Though wastewater treatment plants are distributed all parts of the world they are inefficient to treat bulk quantity of water. So, now major attention has been given to develop efficient and economically feasible adsorbents for contaminants removal from wastewater.

Biochar

In recent years biochar has drawn much attention to be used as an adsorbent substrate. The International Biochar Initiative (IBI) states ‘‘biochar is a solid material obtained from the carbonization of biomass. Biochar has majorly used as a soil amendment as it increases (1) nutrient availability (2) microbial activity (3) soil organic matter (4) water retention and (4) crop yields in soils, while decreasing its (1) fertilizer needs (2) greenhouse gas emissions (3) nutrient leaching and (4) erosion (Sohi et al., 2009; Woolf et al., 2010). Biochar is also having wide range applications due to its stability to physical, chemical and microbiological factors. In recent years it has drawn more attention due to its recalcitrance form of carbon and its role in soil carbon sequestration and climate change mitigation.

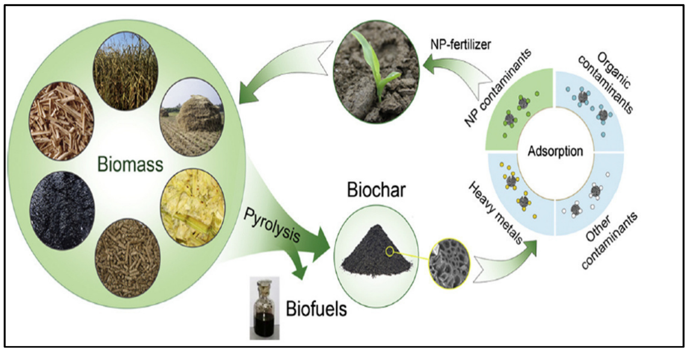
Biochar Role in Wastewater Treatment

The cost-effectiveness of incorporating biochar into conventional granular media filters for water treatment will be determined by its sorption efficiency as well as, its ease of regeneration or replacement and reuse. According to the available literature about biochar application in water treatment, nearly 46% of the studies concerned the removal ability of biochar for heavy metals, 39% for organic pollutants, 13% for NP, and 2% for other pollutants. The specific properties of biochar including large specific surface area, porous structure, enriched surface functional groups and mineral components make it possible to be used as a proper adsorbent to remove pollutants from aqueous solutions. The large specific surface area, porous structure, surface functional groups of biochar are the important features promising their high removal efficiencies of contaminants.



Biochar in Removal of Various Contaminants

Various methods have been employed for the removal of contaminants from wastewater includes adsorption, precipitation oxidation, and reduction, complex formation, and biodegradation. Of which precipitation. Chemical precipitation is the most commonly used method to remove hydroxide, sulfide, carbonate, and phosphate, but sludge production and disposal is a major problem.



Adsorption has evolved as a major treatment method for the removal of all most all the contaminants including for the pollutants which are hard to remove by other methods. Selective adsorption by biological materials, mineral oxides, activated carbon, or polymer resins, has drawn much attention. Activated carbon, often thought of as a universal adsorbent for water treatment, is frequently made from biomass or coal (Mohan and Pittman, 2007). But due to the increased cost of production of activated carbon now biochar is being used as an adsorbent. As an adsorbent, biochar has a porous structure similar to activated carbon, which is the most commonly employed and efficient sorbent for the removal of diverse pollutants from water throughout the world. The presence of cellulose, hemicelluloses, lipids, sugars and proteins in agricultural residue feedstocks contain a variety of functional groups that can be physically activated upon pyrolysis, or by further steam or CO_2 treatment, to improve their ability to adsorb contaminants.

Cao et al. (2009) and Kasozi et al. (2010) reported that Herbicide and pesticide chemicals like atrazine and catechol were reported to sorb on dairy-manure and oak wood biochar, respectively. Chen et al. (2015) used bamboo biochar for adsorptive removal of N-nitroso dimethylamine (NDMA) and gain 50% removal from the water sample. Jung et al. (2013) used loblolly pine biochar for adsorptive removal of pharmaceutical compounds including sulfamethoxazole, diclofenac, carbamazepine, and 17a-ethinylestradiol from aqueous solution. Zheng et al. (2013) examined the effect of biochar particle size on the efficiency of toxic pesticide adsorption. They found that about 54%,51%, 44%, and 31% of atrazine was removed by biochar with various particle sizes of < 0.053, < 0.075, < 0.125, and < 0.250 mm, respectively.

Mechanism Involved in Contaminant Removal

The type and concentration of surface functional groups play important roles in the adsorption capacity of the biochar, and in explaining the adsorbate removal mechanism. Mostly, carbon-structured matrix of biochar, coupled with its high degree of porosity, large surface area, and a strong affinity for non-polar substances such as PAHs, dioxins, furans, and other compounds, enables it to play a vital role as a surface sorbent for controlling contaminants in the environment.

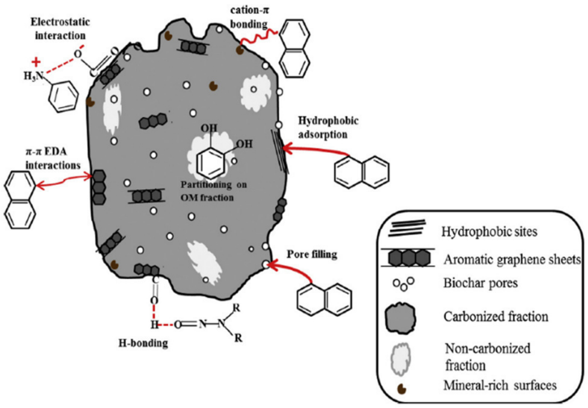


Fig. 2. Sorption mechanisms for the uptake of organic contaminants on biochar.

The surface of biochar can contain an abundance of chemically active groups, (e.g. OH, COOH, and ketones, etc.) that bestow biochar with an immense potential to adsorb heavy metals and toxic substances, such as aluminum (Al) and manganese (Mn) in acid soils, and arsenic (As), cadmium (Cd), copper (Cu), nickel (Ni) and lead (Pb) in heavy metal contaminated soils. Cu(II) and Pb(II) were removed using surface complexation with carboxylic and hydroxyl groups, respectively, whereas Al(III) was more generally removed using oxygen-containing groups.

Most of the heavy metals were adsorbed on the biochar surface through inorganic constituents in the biochar. Organic contaminants were either removed by sorption, or interaction with functional groups and surface charges. Due to the dissociation of oxygen-containing functional groups, biochar mostly carries net negative charges on their surfaces. Consequently, they can be utilized as low-cost adsorbents for the removal of organic contaminants and heavy metal cations from water.

References

- Cao, X., Ma, L., Gao, B., Harris, W., 2009. Dairy-manure derived biochar effectively sorbs lead and atrazine. *Environ. Sci. Technol.* 43, pp.3285–3291.
- Chen, C., Zhou, W., Lin, D., 2015. Sorption characteristics of N-nitroso dimethylamine onto biochar from aqueous solution. *Bioresour. Technol.* 179, pp.359–366.
- Jung, C., Park, J., Lim, K., Park, S., Heo, J., Her, N., Oh, J., Yun, S., Yoon, Y., 2013. Adsorption of selected endocrine disrupting compounds and pharmaceuticals on activated biochars. *J. Hazard. Mater.* 263 (Pt 2), pp.702–710.
- Zheng, H., Wang, Z., Zhao, J., Herbert, S., Xing, B., 2013. Sorption of antibiotic sulfamethoxazole varies with biochars produced at different temperatures. *Environ. Pollut.* 181, pp.60–67.
- Inyang, M. and Dickenson, E., 2015. The potential role of biochar in the removal of organic and microbial contaminants from potable and reuse water: a review. *Chemosphere*, *134*, pp.232-240.
- Mohan, D., Sarswat, A., Ok, Y.S. and Pittman Jr, C.U., 2014. Organic and inorganic contaminants removal from water with biochar, a renewable, low cost and sustainable adsorbent–a critical review. *Bioresource technology*, *160*, pp.191-202.
- Tan, X., Liu, Y., Zeng, G., Wang, X., Hu, X., Gu, Y. and Yang, Z., 2015. Application of biochar for the removal of pollutants from aqueous solutions. *Chemosphere*, *125*, pp.70-85.

6. Role of Biochar in Soil Carbon Sequestration

Vidya Shree Bharti & Vijay Kumar A
ICAR- Central Institute of Fisheries Education, Versova, Mumbai-400061

Emissions of GHGs

Carbon dioxide (CO_2) is one of the most potent GHGs, accounting for nearly 60% of the overall radiative forcing of the atmosphere. Global atmospheric CO_2 concentration has increased to 405 ppm in 2017, exceeding the pre-industrial levels by about 40% (World Meteorological Organization, 2018) with a steady increase of approximately 0.50% per year over the past century (IPCC, 2013). World agriculture accounted for an estimated emission of 5.1–6.1 Pg CO2 equivalents year contributing 10–12% to the total global anthropogenic GHG emissions in 2005. Methane (CH_4) is second only to CO_2 in volume but has 72 times more global warming potential than CO_2 on a mass basis. CH_4 and CO_2 together, contribute to approximately 80% of the overall radiative forcing of the atmosphere due to anthropogenic influence (Myhre *et al*., 2013).

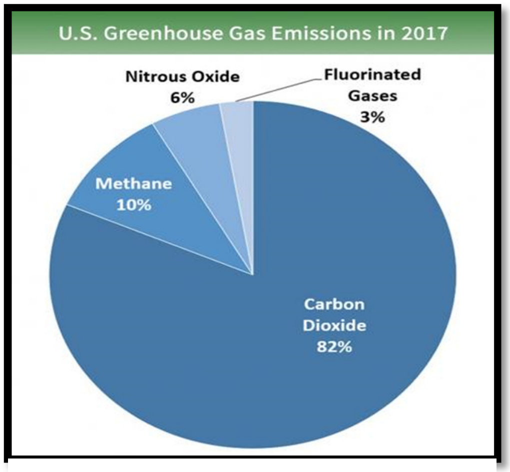


Fig. 1: Emissions of different GHGs

The increased level of GHGs already increased the average global temperature of the earth by 1ºC above the pre-industrial baseline and 2015-18 are the four warmest years of the record (World Meteorological Organization, 2018). The International Panel on Climate Change (IPCC) predicts that, by end of the 21st century, the atmosphere may contain up to 570 ppm CO_2, causing a rise in the mean global temperature of around 1.9ºC. This drastic increment in global climate change causes extreme weather events, prolonged periods of drought and floods, shifting of climatic zones, uneven rainfall, sea level is expected to rise between 0.19 and 0.58 meters.

Carbon Sequestration

To tackle this drastic phenomenon, there was much attention towards reducing the emissions of GHGs, and also to sequester CO_2 in longer pools. The Paris Climate Agreement recommended a voluntary plan of "4 Per Thousand" (4PT) to sequester C in world soils at the rate of 0.4% annually to 0.4 m (1.3 ft) depth (UNFCC 2015). The basic process of C sequestration in the terrestrial biosphere involves the transfer of atmospheric CO_2 into plant biomass through photosynthesis and conversion of biomass into stable SOC through formation of organo-mineral complexes. Carbon sequestration is an important strategy in combating rising carbon dioxide concentration in the atmosphere. There is much attention is given towards increasing the soil carbon sequestration potential and adopting proper management practices.

Soil as a Major Sink for CO_2

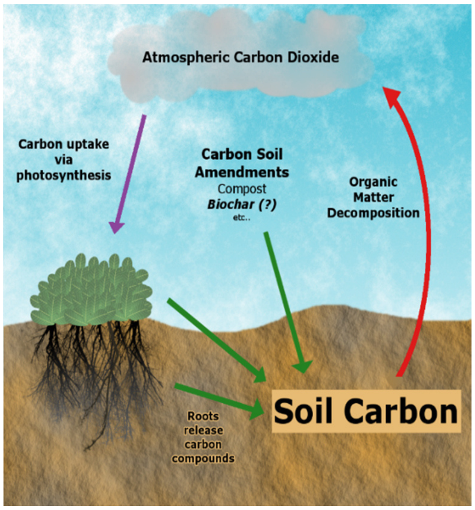


Fig. 2: Soil carbon sequestration

Soil is the largest terrestrial carbon sink which contains 1100–1600 Pg. sequestered C which is more than twice the amount of C in vegetation (560 Pg.) and thrice the amount of carbon in the troposphere (750 Pg.) (Brar *et al*., 2013). The majority of the soil C held in the form of Soil organic carbon (SOC) which is an important component for the functioning of various ecosystems and also for maintenance of soil health which intern helps in improve and sustain food production. SOC has a major influence on soil structure, water holding capacity, cation exchange capacity, nutrient retention, control of soil erosion and nutrient leaching. The increase of soil organic carbon can be done by the supply of soil organic matter in the form of organic wastes, manures, and crop residues. This is assumed that SOM comprises 58% C which upon microbial mineralization adds to SOC and improves the soil physical (soil structural stabilization), chemical (buffering and changes in soil pH), and biological properties (substrate and supply of nutrients for microbes).

Role of Biochar on Soil Carbon Sequestration

Biochar is the carbonaceous product obtained by the thermal degradation of organic materials in the absence of oxygen. Biochar is the ‘‘charred organic matter, produced with the intent to deliberately apply to soils to sequester C and improve soil properties (Lehmann and Joseph, 2009). Biochar is a highly porous, carbon (C)-rich solid residue of pyrolysis of

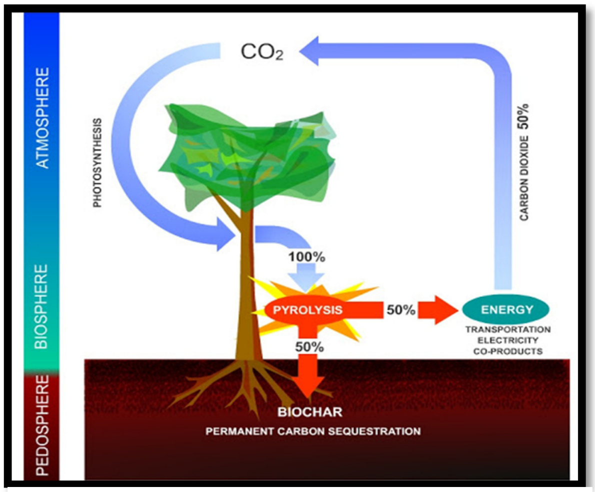


Fig. 3: Role of biochar on soil carbon sequestration

biomass and has recently been suggested for applications in the soil as a means of mitigating global climate change. Biochar application has been shown as one promising means of reducing the atmospheric CO_2 concentration slowing down the rate at which photosynthetically fixed carbon (C) is returned to the atmosphere and also reduces the emissions of other greenhouse gases. Biochar apart from acting as a stable form of carbon it also known for its improvement of soil nutrient retention and water holding capacity and also acts as buffering agent and helps in the improvement of soil productivity and helps in enhanced photosynthesis activity and carbon fixation.

Biochar's climate-mitigation potential primarily stems from its highly recalcitrant nature towards chemical and microbial oxidation and will persist in soil for hundreds to thousands of years. Biochar is a highly heterogeneous material with a chemical composition that varies widely depending on feedstock and pyrolysis conditions. The decomposition rate of biochar varies significantly with experimental durations, feedstocks, pyrolysis temperatures, soil pH and clay contents. Biochar produced at lower temperatures were reported to mineralize faster than those produced at higher temperature with increased aromaticity and degree of aromatic condensation.

Mohan *et al.* (2018) synthesized biochar from Corn Stover and rice husk biomass at 550 and 650ºC respectively, and he observed their effects on CO_2 efflux from the soil system. The application of both biochar's (Fig. 4) significantly reduced the emissions of CO_2 compared to control and only biomass application and also higher reduction was observed at higher concentrations of biochar. Similar work conducted by Bhaduri *et al.* (2016)

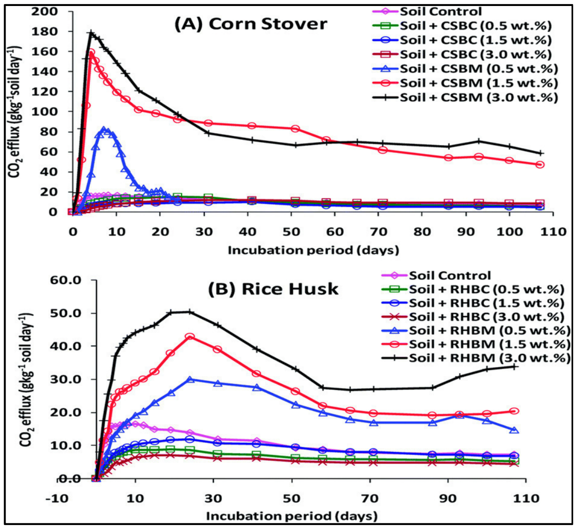


Fig. 4: Effect of Corn Stover and Rice husk biochar on CO_2 efflux from soil

used peanut shell biochar at the rate of 0, 2.5%. 5% and 10% w/w of saline soil and reported that biochar could increase soil organic carbon on the application of highest rate of biochar addition (10%), and potentially restore the saline soils by less C mineralization and more sequestration of soil carbon.

References

- Bhaduri, D., Saha, A., Desai, D. and Meena, H.N., 2016. Restoration of carbon and microbial activity in salt-induced soil by application of peanut shell biochar during short-term incubation study. *Chemosphere*, *148*, pp.86-98.
- Brar, B.S., Singh, K. and Dheri, G.S., 2013. Carbon sequestration and soil carbon pools in a rice–wheat cropping system: effect of long-term use of inorganic fertilizers and organic manure. *Soil and Tillage Research*, *128*, pp.30-36.
- Lehmann, J., Joseph, S., 2009. Biochar for environmental management: an introduction. In: Lehmann, J., Joseph, S. (Eds.), Biochar for Environmental Management: Science and Technology. Earthscan, London, pp. 1-12.
- Mohan, D., Abhishek, K., Sarswat, A., Patel, M., Singh, P. and Pittman, C.U., 2018. Biochar production and applications in soil fertility and carbon sequestration–a sustainable solution to crop-residue burning in India. *RSC advances*, *8*(1), pp.508-520.
- UNFCC (United Nations Framework Convention on Climate Change). 2015. COP21 Climate Agreement. Paris, France: United Nations Framework Convention on Climate Change.

7. Biochar for Pollutant Removal with Special Reference to Emerging Pollutants

Saurav Kumar & Ashutosh Kumar Singh
ICAR- Central Institute of Fisheries Education, Versova, Mumbai-400061

Introduction

Biochar is the most common ingredient and technology used in agriculture sector for improving soil nutrient and carbon content and defined as a carbon rich solid which is obtained by heating or pyrolysis of biomass such as wood, solid natural plant part, manure with little or no oxygen. Biochar can even reduce carbon release to the atmosphere due to burning or degradation by carbon stabilization into a form resembling charcoal. Biochar is comparatively similar to activated carbon in terms of porosity which is most commonly employed and efficient sorbent for removal of diverse pollutant from water throughout the world. But the production cost of biochar is lower as it activated carbon requires higher temperature and additional process for its production. However, biochar is differ from activated carbon or charcoal in their preparation method and chemical nature but both have majority carbon content. Soil carbon content and its properties can be improved by burying it in fields. The energy produced from pyrolysis process can also be potential substitute for fossil fuels. Biochar can be even used as adsorbent to remove pollutant from aqueous solution as it has large specific area, porous structure, enriched functional groups and mineral components. The feedstock for biochar production are in abundance and is of low-cost as it is mainly obtained from agricultural biomass and solid waste. The invasive plants can even be converted into biochar, which can additionally improve invasive plant management and also protect environment from its negative effects. The conversion of biomass into biochar and its usage as a sorbent can perform dual role of improvement in waste management and protection of environment.

Application of Biochar

Biochar in Animal Farming

Biochar can be used in feeding, litter or in slurry treatment. The main advantage of biochar usage in animal farming are-:

- Used as a feed supplement in animal farming

- Used to control incidence of diarrhoea
- Used to improve feed intake
- Used to control allergies in animal

Biochar as Soil Conditioner

Biochar have positive effect on soil fertility when used in soil. The various effects are-:

- Increase in water holding capacity of the soil
- Increase in air content of the soil
- Release of nutrients through raising the soil's pH-value
- Used as organic based fertiliser by mixing biochar with such organic waste as wool, molasses, ash, slurry and pomace
- Prevent leaching of nutrients
- Positive effect on plant defense mechanism

Recently the effect of biochar on fish health especially growth and immune system was also revealed the potential use of biochar as feed supplement in aquaculture. However a thorough research data need to be generated to prove the viability of technology in various aquatic system and fish species.

Biochar as Construction Material

The two interesting properties of biochar are its extremely low thermal conductivity and its ability to absorb water up to six times its weight. These properties mean that biochar is just the right material for insulating buildings and regulating humidity. In combination with clay, but also with lime and cement mortar, biochar can be added to clay at a ratio of up to 50% and replace sand in lime and cement mortars. This creates indoor plasters with excellent insulation and breathing properties, able to maintain humidity levels in a room at 45–70% in both summer and winter. Biochar is an efficient adsorber of electromagnetic radiation, meaning that biochar-mud plaster can prevent "electrosmog". Biochar can also be applied to the outside walls of a building by jet-spray technique mixing it with lime. Applied at thicknesses of up to 20 cm, it is a substitute for styrofoam insulation. Houses insulated this way become carbon sinks, while at the same time having a more healthy indoor climate.

Biochar for Water Treatment

The important features of biocahar like large specific surface area, porous structure and surface functional groups are leading to the high removal efficiencies of contaminants. The contaminants include heavy metals, organic pollutants and other pollutants. According to the available literatures nearly 46% of the studies concerned the removal ability of biochar for heavy metals, 39% for organic pollutants, 13% for NP, and 2% for other pollutants.

Removal of Heavy Metals

The abundant surface functional groups (mainly oxygen-containing groups, e.g. carboxylate, COOH; and hydroxyl, OH) existing on biochar's surface, have a strong interactions with heavy metals such as electrostatic attraction, ion-exchange and surface complexation. These effects are the determining factor for removal of heavy metals from water.

Organic Pollutants

The adsorption mechanisms by which organic contaminants bind to biochars are also combined with different kinds of interactions. These interaction are electrostatic interaction, hydrophobic effect, hydrogen bonds, and pore-filling may be the main mechanisms for the adsorption of organic contaminants onto biochar.

Persistent Organic Pollutants (POPs)

POPs are extremely difficult to decompose in the natural environment and will persist in the water environment, soil and food cycle for a long time, and still exist in the human body for a long time. Straw-based biochar and wood-based biochar are extremely effective in removing POPs from aqueous solutions (Shi et al., 2016).

Emerging pollutants and application of biochar for removal efficacy

The mushrooming of industries leads in the development of new chemical compound to improve our quality of life which results in discharge of emerging Pollutants (EPs) into aquatic environment. Between 2002 and 2011, over 50% of the total production of chemicals is represented by environmentally harmful compounds and more than 70% of these are chemicals with significant ecological impact (EUROSTAT, 2013). The quality of the aquatic environment depends deeply on both natural processes and anthropogenic activities. In recent decades, with the advancement of technologies, it has been possible to detect and analyse several trace pollutants, but still a wide array of undetected emerging contaminants of environmental concern need to be identified and quantified (Gavrilescu *et al.,* 2015). These pollutants may be mobile and persistent in different soil-water matrices and can exhibit relative effects to both human and aquatic organisms even at low concentrations (nanogram level). Detrimental effects of EPs include alteration of physiological response in fishes by causing the haematological, enzymological, genotoxicity, endocrine disruption, and immune toxicity. Emerging pollutants in aquatic environment includes pharmaceuticals, personal care products, endocrine disrupting hormones, antibiotics used in aquaculture, Artificial Sweeteners (ASs), disinfection by-products, Surfactants, metals, pesticides, persisting organic compounds, other agrochemicals and algal toxins. Therefore there is a need to discover a novel technology to remove these EPs from aquatic system.

Factors affecting adsorption of contaminants on biochar

The adsorption efficiency of biochar tend to be influenced by properties of biochar, deashing treatment, pH, dosage of adsorbent, competitive anions, and temperature.

Properties of Biochar

The pyrolytic temperature, residence time, feedstock, and thermochemical conversion technology have profound effects on the properties of biochar. These properties in turn influence on the adsorption efficiency toward various pollutants. Among which, temperature is presumably a key parameter. Temperature influence on structural characteristics and isotherms shape of the biochar. Chen et al. (2012) stated that the pyrolytic temperature of a biomass affected the rate of uptake of a compound by a biochar because the temperature affected the degree of carbonization of a biochar. At higher temperature, organic matters gets completely carbonized, the surface area gets increased, and more nanopores are developed, resulting in the sharply enhanced adsorption rate.

De-mineralization and Deashing Treatment

These treatments factor have significant influence on the adsorption property of biochar. This is mainly because that deashing can greatly influence the composition and the surface characteristics of the biochar. All surface element contents decreased after deashing except for carbon. Zhang et al. (2013a) stated that adsorption increased greatly on the deashed biochar due to removal of inorganic compounds, which blocked some organic adsorption sites in the original.

Solution pH

The solution pH is one of the most vital parameters in the optimization of adsorption process. The influence of pH on adsorption depends on types of biochars and the target contaminants. It affects not only the adsorbent surface charge, but also the degree of ionization and speciation of the adsorbate. Biochar carried various surface functional groups (mainly oxygen containing groups, (e.g. carboxylate, COOH; and hydroxyl, OH). The behavior of these functional groups change with the increase of the solution pH. At low pH, most of these functional groups present on biochars are protonated and presented in positively charged form. For pH < pHpzc (point of zero charge), the biochar surface is positively charged, favoring adsorption of the anions (Abdel- Fattah et al., 2014). In addition, the presence of a large number of H^+ and H_3O^+ in the aqueous solution may compete with the cation for adsorption sites available on biochars. Thus electrostatic repulsion will occur between cation contaminants and positively charged biochars surface thus a lower adsorption can be observed at low pH. With the increase of pH value, the competition of metal ions and protons for binding sites decreased and more binding sites are released due to the deprotonation of functional groups (Lu et al., 2012). The surface of biochar is negatively charged when pH > pHpzc. Therefore, in the higher pH range, the cations can be easily captured by biochar surface.

Co-existing ions

The co-existence of ions exert significant influence on the equilibrium adsorption capacity, especially for the application of biochar in the real water system, because complex pollutants usually co-exist in environment, and have interaction effects on the adsorption efficiency. The studies of adsorption co-existed ions can aid in a better understanding of biochar adsorption mechanism of contaminants in the environment. Jia et al. (2013) investigated the effects of metal ions on the adsorption of oxytetracycline (OTC) by maize straw derived biochar. It turned out that the presence of heavy metals had different effects on OTC adsorption to biochar: insignificant by Cd^{2+}, slight facilitation by Zn^{2+}, slight inhibition by Pb^{2+}, and Cu^{2+} facilitated the adsorption of OTC at all measured pH values.

Dosage of Adsorbent

The dosage of adsorbent has significant influence on the adsorption efficiency. Applying an optimum dosage of biochar to contaminants removal is crucial for its cost-effective application. Tsai and Chen (2013) found that increasing the adsorbent concentration did result in an increased removal efficiency of the total heavy metals due to the increase in total amount of active sites.

Temperature

Most previous studies had reported that the adsorption of contaminants by biochars appeared to be an endothermic process and the adsorption capacity increased with increasing temperature (Zhang et al., 2013b). Meng et al. (2014) studied the effect of temperature (at 15, 25 and 35 ^{O}C) on Cu(II) adsorption onto biochar derived from swine manure, and reported that adsorption capacity increase with increase in temperature.

Conclusion

Biochar is gaining attention as a low-cost, high-efficiency, renewable adsorbent. The methods of preparation of biochar is ecologically safe, sustainable. It can be a better adsorbent for removal of pollutants by optimising the removal process. Biochar holds the promise to tackle chronic human development issues like hunger and food insecurity, low agricultural productivity and soil depletion, deforestation and biodiversity loss, energy poverty, water pollution, air pollution and climate change.

References

- Abdel-Fattah, T.M., Mahmoud, M.E., Ahmed, S.B., Huff, M.D., Lee, J.W., Kumar, S., 2014. Biochar from woody biomass for removing metal contaminants and carbon sequestration. J. Ind. Eng. Chem. http://dx.doi.org/10.1016/j.jiec.2014.06.030.
- Chen, Z., Chen, B., Chiou, C.T., 2012. Fast and slow rates of naphthalene sorption to biochars produced at different temperatures. Environ. Sci. Technol. 46, 11104–11111.
- Jia, M., Wang, F., Bian, Y., Jin, X., Song, Y., Kengara, F.O., Xu, R., Jiang, X., 2013. Effects of pH and metal ions on oxytetracycline sorption to maize-straw-derived biochar. Bioresour. Technol. 136, 87–93.
- Lu, H., Zhang, W., Yang, Y., Huang, X., Wang, S., Qiu, R., 2012. Relative distribution of Pb2+ sorption mechanisms by sludge-derived biochar. Water Res. 46, 854–862

- Meng, J., Feng, X., Dai, Z., Liu, X., Wu, J., Xu, J., 2014. Adsorption characteristics of Cu (II) from aqueous solution onto biochar derived from swine manure. Environ. Sci. Pollut. Res. 21, 7035–7046.
- Tsai, W.T., Chen, H.R., 2013. Adsorption kinetics of herbicide paraquat in aqueous solution onto a low-cost adsorbent, swine-manure-derived biochar. Int. J. Environ. Sci. Technol. 10, 1349–1356.
- Zhang, P., Sun, H., Yu, L., Sun, T., 2013a. Adsorption and catalytic hydrolysis of carbaryl and atrazine on pig manure-derived biochars: impact of structural properties of biochars. J. Hazard. Mater. 244, 217–224.
- Zhang, Z.B., Cao, X.H., Liang, P., Liu, Y.H., 2013b. Adsorption of uranium from aqueous solution using biochar produced by hydrothermal carbonization. J. Radioanal. Nucl. Chem. 295, 1201–1208.
- Shi, K., Qiu, Y., Li, B., Stenstrom, M.K., 2016. Effectiveness and potential of straw and wood-based biochars for adsorption of imidazolium-type ionic liquids. Ecotoxicol. Environ. Saf. 130, 155e162.

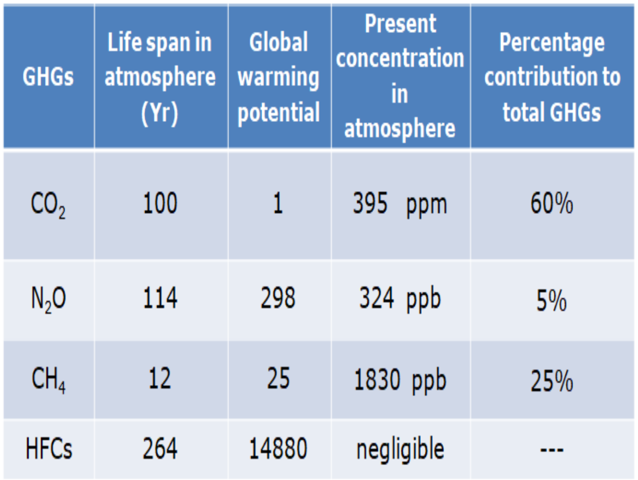
8. Green House Gases (GHGs) Emissions from Aquaculture Systems and it's Mitigation

Vidya Shree Bharti & Chittranajan Raul
ICAR- Central Institute of Fisheries Education, Versova, Mumbai-400061

Introduction

Global warming and climate change have emerged as major threats to the world community in recent times. Natural disasters are becoming more intense and frequent in countries around the world. The effects of global warming are evidently observed in terms of melting polar ice, sea-level rise, irregular rainfall, ocean acidification, tropical cyclone, forest fires, floods, drought, biodiversity loss, etc. Global warming is caused by an increase in the amount of long-lived GHGs in atmosphere, having the capacity to trap heat (longwave radiations) and keep earth's lower atmosphere warmer. The emission of three long-living GHGs (CO_2, CH_4, and N_2O) from the anthropogenic sector contributes more than 90% global warming effect. The present atmospheric concentration of CO_2, CH_4, and N_2O are 395 ppm, 324 ppb, and 1830 ppb respectively (IPCC, 2015). N_2O has the highest global warming potential, which is about 310 times than CO_2. China contributes the highest anthropogenic GHGs to the total global emission of about 11.1 Gt CO_2 eq., followed by USA and EU. India is placed at 4th position with a contribution of 2.9 Gt CO_2eq. GHGs emission (WRI, 2014). Major emissions are contributed by the sectors like industry, electricity & heat production, agriculture, forestry, building, transportation, and other sectors. The agriculture and allied sectors of the world share 24% of total emissions, but there is no concrete data for aquaculture sectors' contribution to GHGs emissions. Aquaculture is the fastest-growing sector with an annual growth rate of approximately 6% and per capita fish consumption of 20kg. It provides food, nutrition, and employment to 100 million of people. From the total worlds fish production (170.9 MMT) aquaculture contributes 80 MMT and rest are from capture fisheries (SOFIA, 2018). Globally the annual utilization of compounded feed is 1 billion tons for all livestock and fisheries, from which annually 16.6 million tons of carbon buried in the aquaculture industry in the form of aquafeed (IFIA, 2019). As the fish feed is protein-rich, the leftover feed and faecal matter are the ultimate sources for the release of nitrous oxide, carbon dioxide, and methane gases during the process microbial mineralization. Therefore the aquaculture sector is also responsible for global warming by emitting GHGs, but the actual figure is unknown.

Different world organizations are conducting research for quantifying GHGs from the aquaculture sector and its possible mitigation.



GHGs	Life span in atmosphere (Yr)	Global warming potential	Present concentration in atmosphere	Percentage contribution to total GHGs
CO_2	100	1	395 ppm	60%
N_2O	114	298	324 ppb	5%
CH_4	12	25	1830 ppb	25%
HFCs	264	14880	negligible	---

Table.1. Lifespan, Global warming potential, atmospheric concentration of GHGs.

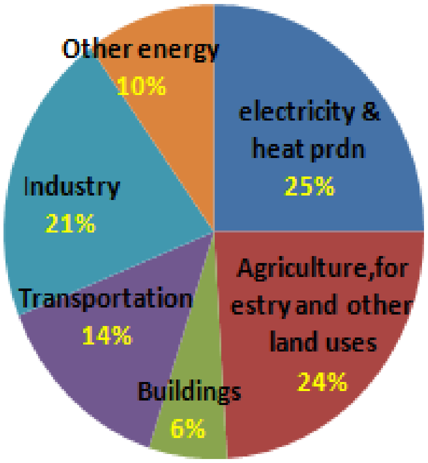


Fig.1. Global Sector wise GHGs emission (IPCC, 2014).

Process of GHGs emission from aquaculture system

Emission of Carbon dioxide (CO_2)

The source of CO_2 production in aquaculture systems are respiration by biological components (fishes, phytoplankton, benthic organism, microorganism) and mineralization of organic matter (Chen et al. 2015). The heterotrophic bacteria mainly produce CO_2 during the mineralization of autochthonous/ allochthonous organic matter. The feed residues and the faecal matter of fishes are the major sources of organic matter. Manure applications, as well as autochthonous dead phytoplankton biomass, also contribute to the organic matter content in most of the aquaculture system. The emission of CO_2 depends upon many factors like air and water temperature, pH, rate of algal productivity and partial pressure of CO_2 in water and surface-atmosphere. The negative flux (absorption from the atmosphere to water) of CO_2 frequently occurs as it has a high solubility in the water when the partial pressure of CO_2 in water is very low (In high alkaline pH of water, the CO_2 gas transforms into bound CO_3^{2-} form). In acidic pH, the high flux of CO_2 occurs from water to the atmosphere (Yang *et al.,* 2015). There is a direct relationship between water temperature and carbon dioxide flux in aquatic systems. As CO_2 is the principal nutrient source for algae/ phytoplankton productivity, the flux reduces depending on the spatial and temporal productivity of primary producers.

Emission of Methane (CH_4)

The methanogenic bacterium produces methane gas by utilizing dissolved organic carbon (DOC) in anaerobic conditions. In the aquaculture system, the pond bottom sediment is the major site for methanogenic bacteria activity as it resides at the least aerated site of the pond environment. Methanogenic bacteria utilize DOC as a substrate for energy and biomass production. The DOC produces in the process of organic matter mineralization by heterotrophic bacteria, churning of sediment by micro/macro-benthic organisms and some extent from the faecal matter of detritivorous fish. The emission of methane gas from the aquatic system to the atmosphere occurs by the method of ebullition (in the form of gas bubbles) and diffusive flux. The CH_4 flux from the aquaculture system depends on water and air temperature, which indicates that CH_4 emission relates to the water thermal regime. An increase in temperature stimulates methanogenesis activities, which would contribute to higher CH_4 emission. There are many reports of seasonal variation in CH_4 emission, with the emission being on the higher side in summer and lower in autumn, winter and spring (Hu*et al.,* 2016).The flux of methane also depends on the dissolved oxygen concentration of the aquaculture system, because aerobic condition inhibits the activity of methanogens.

Emission of Nitrous Oxide (N_2O)

The nitrifying and denitrifying bacteria produce nitrous oxide gas through autotrophic aerobic nitrification, anaerobic denitrification, ANNOMAX process. These bacteria utilize the total ammonia nitrogen (TAN) (NH_3& NH_4^+), which releases from the degradation of the uneaten protein (source of nitrogen element) rich aqua feed and faecal excreta of fishes. Many studies provide information that algal photosynthesis also releases NO_3^- and dissolved oxygen, which increases the proportion of N_2O production coupled with denitrification and nitrification (Yang *et al.,* 2015). As the nitrogen cycle is very complex and it differs on the basis of natural microbial flora, 90% of N_2O is produced in the denitrification process (occurs in anaerobic condition) and rests from the nitrification process (occurs in aerobic condition). Aquaculture pond sediment pH plays a major role in the production of nitrous oxide. The acidic pH conditions of sediment inhibit the reductase activity of N_2O (preventing further oxidation by denitrifiers) which causes the emission of more N_2O during anaerobic denitrification process.

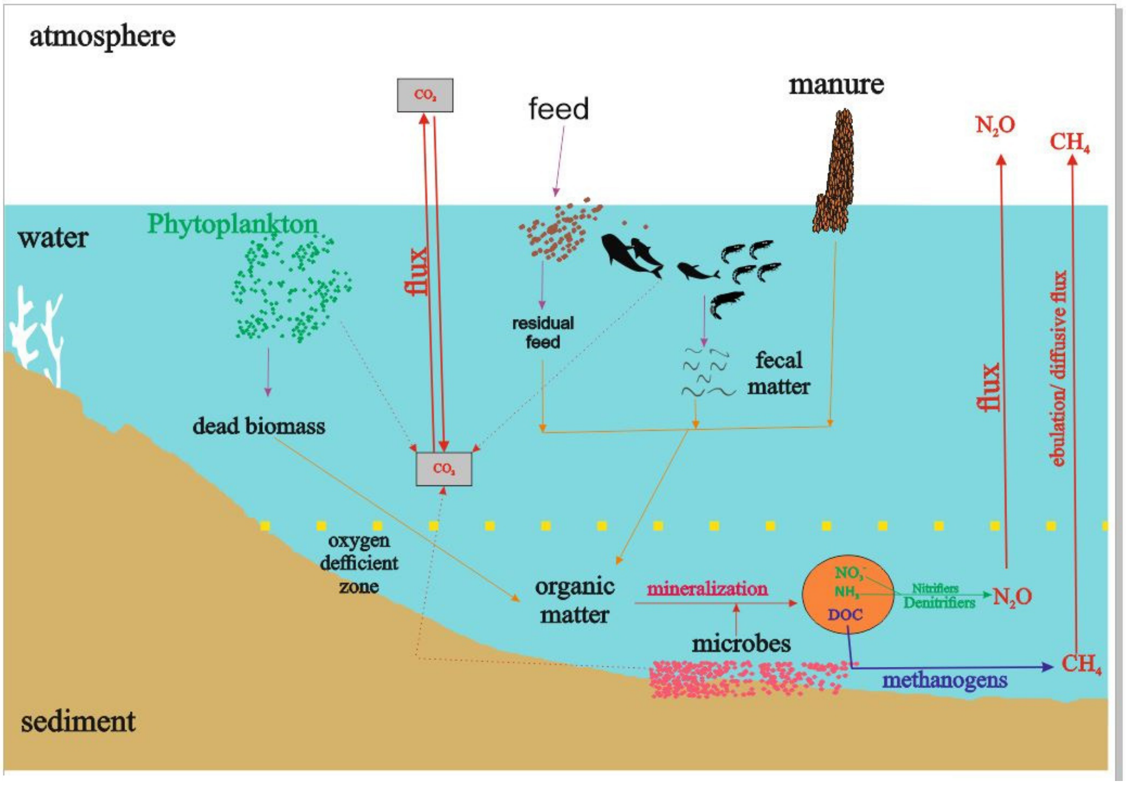


Fig.2. Process of GHGs emission from aquaculture system

Emission Status of Aquaculture systems

Till now there is no clear-cut figure of total GHGs emissions from the aquaculture sector and individual data of different culture systems. The data are available for area wise culture systems, species-specific culture systems, and emissions from water bodies of different countries. The emission from aquaculture in 2009 is estimated to be 9.30×10^{10} g eq. CO^2 and will increase to 3.83×10^{11} g eq. CO^2 which accounts for 5.72% of anthropogenic N_2O-N emission by 2030 (Hu et al., 2012). FAO reported that there are 87500 km^2 of freshwater aquaculture pond resources available worldwide, from which the temperate monsoon climate regions aquaculture ponds contribute an estimated 2.98 million t yr^{-1} CO_2, which is 0.0088% of the current annual global CO_2 emissions. Based on estimates from recent research, global freshwater environments could be emitting 1.2-2.1 Pg of CO_2yr^{-1} and 0.65 Pg of CH4 yr^{-1}(Chen et al., 2015). The emissions from the aquaculture system from different research have been given in Table no 2 as follows.

Table2. Emissions of GHGs from different Aquaculture system.

Culture System	**Country**	**CO_2 emission**	**CH_4 emission**	**N_2O emission**	**CO_2eq. emission**	**Reference**
Carp culture	India				1.84 kg CO_2eq/kg LWF	Robb *et al.*,2017
Nile tilapia	Bangladesh				1.58 kg CO_2eq/kg LWF	Robb *et al.*,2017
Stripped catfish culture	Vietnam				1.37 kg CO_2eq/kg LWF	Robb *et al.*,2017
Composite fish culture	India	1056 -1758 (g/ha/day)	3.62 - 7.86 (g/ha/day)	0.31-1.11(g/ha/day)	0.096 CO_2 eq. kg/kg	(Srinivasa et al.,2015-16
(Shrimp culture) *P momodon &* *L vennamai*	India	1.88-5.14 (g/ha/day)	1.88 -5.14 (g/ha/day)	0.07-0.35 (g/ha/day)	0.04 CO_2eq.kg/ kg fish	(Srinivasa et al.,2015-16
Sea bass	India				0.02 -0.03 CO_2 eq.kg/kg fish	(Srinivasa et al.,2015-16
Shrimp	Chinna	20.78 $mgm^{-2} h^{-1}$	19.95mg $m^{-2}h^{1}$	10.74$mgm^{-2} h^{-1}$		Yang *et al.,* 2015
Polyculture	Chinna	-60 $mgm^{-2} h^{-1}$	1.65 $mgm^{-2} h^{-1}$	11.8$mgm^{-2}h^{-1}$		Yang *et al.,* 2015
Shrimp ppond	NE Brasil				1,390 Mg CO_2 e/ha	Kauffman *et al.,* 2018
Crab pond	SE Chinna		1.140.50 $mgm^{-2}h^{-1}$			Hu *et al.,* 2016
Grass carp plyculture	Chinna	50.6 to 151.0 mg/ m^2/hr				Chen et al.,2015
Shrimp pond floor	Indonesia	1.60 kg CO_2 $m^{-2}y^{-2}$				Sidik*et al.,* 2013
Shrimp farm in Mangrove	Brasil	26.5$mgm^{-2} h^{-1}$	40.4 $\mu gm^{-2}h^{-1}$			Queiroz et al., 2019

Mitigation Strategies for GHGs emission from Aquaculture system

Among the three long-lived GHGs, methane and nitrous oxide draws more attention, because the global warming potential is much higher and very difficult to sequester after releasing to the atmosphere as compared to carbon dioxide. The atmospheric CO_2 can be sequestered as blue carbon (plant biomass). The possible mitigation strategies for reduction of GHGs from the aquaculture system are as follows;

Preventing of Aquaculture Practices in Carbon Sequestered Sites-

The ecological sensitive sites like mangrove, salt marsh, estuarine area, and wetlands are the natural sites for carbon sequestration. Huge quantities of carbon and nutrients get sequestered in these sites from terrestrial runoff and also from the autochthonous dead biomass. Many countries (especially developing countries) are disturbing these sites by practicing commercially important shrimp and fish aquaculture for generating high profit. The disturbances create havoc by destroying the concerned area with greater emission of GHGs, from the carbon and nutrient, which are already stored in the system. Many research findings show higher emissions of CO_2, CH_4, and N_2O in sites converted into the aquaculture system. Countries should have strict legislation to ban intensive aquaculture practices in carbon sequestered areas and encouraging aquaculture in degraded soil environments like inland saline areas where the carbon pool is very less.

Adopting Advanced Scientific Aquaculture Practices-

Practising Herbivorous Fish Polyculture System

Commercial aquaculture of carnivorous fishes mostly comprises of protein-rich formulated feed supplement, which is the major contributor for GHGs formation. In herbivorous fish polyculture system, the candidate species selected on the basis of feeding practice, which feeds upon phytoplankton, algae, and submerged weeds in different depth of pond water. The primary producers utilize the nutrients (CO_2, NO_3^-) preventing the GHGs substrate for microbes. The bottom submerged weed and detritivorous fishes (churning of sediments) create aerobic conditions and positive redox potential in the bottom sediment environment. It leads to a decrease in the activity of methanogens and denitrifiers. In this aquaculture system, the formulated feed supplementation is relatively less. It will also reduce the input of fish meal to aquaculture practices, hence making the feed cost-effective.

This system should be attempted in all countries for better utilizing of pond bottom sediments.

Integrated Multi tropic Aquaculture (IMTA)

The system comprises species of each tropic level having different requirements of feed like finfish, shellfish, bivalve, and seaweed. The waste generated from one tropic level component serves as feed for others in the same system and finally, all the inorganic waste (CO_2, TAN) nutrients are taken by seaweeds. In this process, the complete waste utilization loop is formed which minimizes the emissions from the aquaculture system. Systems like seaweed fish polyculture are already practiced in many countries as a component of coastal aquaculture and cages culture. IMTA could be established thereby selecting candidate species for different tropic levels.

Aquaponics

This is a hybrid system of aquaculture and hydroponics. In this system, the fish culture unit and plant farming unit remain in two separate systems or in a combined system. Fishes like Tilapia, Pangasius, which utilize the feed maximally and produce more waste that is required for the growth of foliage plants like Spinach, and other leafy vegetables can intensify the culture system with high production and better output without harming the environment. The high stocking density fish culture which produces nutrient-rich wastewater (CO_2, NH_4^+, and NO_3^-). utilized by plant for growth hence reduces the emission of GHGs by ultimate sequestration as plant biomass.

Using of Fish Feed Additives

The yucca plant extract feed additive contains saponin which is the major bioactive component present in the steroidal form (Mao et al., 2010). This component reduces the level of free ammonia (substrate for GHGs for nitrifying and denitrifying bacteria) by physically binding to ammonia. It also reduces methane emission by controlling the protozoa population as saponin damages the cell wall. This is because protozoa harboris an active population of methanogenic archaea both on their external and internal surfaces. The Yucca extract can be used to improve water quality by reducing the concentration of total ammonia nitrogen and nitrate in fresh and marine water used for aquaculture. From the total studies on livestocks, 31% are conducted in aquatic species like pacific white shrimp, Nile tilapia, channel catfish, *Marsupenaeusjaponicus* , etc (Adegbeye et al., 2019). Yucca can reduce methane, total ammonia nitrogen, and nitrous oxide in water to a range of 8.49%-69%, 50%-100%, and 75% respectively. So it is a potential asset in the face of global warming-induced climate changes.

Pond Bottom Sediment Management

The pond bottom sediment is the chemical laboratory for all biochemical degradation of carbon substrate and production of GHGs. The anoxic condition of pond sediment and negative redox potential favour the activity of methanogenic bacteria and denitrifiers. The application of biochar to bond bottom sediment prior to culture practice can reduce GHGs emissions significantly. Biochar is the pyrolysis product of any organic or agricultural waste material at higher temperatures ranges from 300 to 700℃ (Raul et al., 2019). Though till now there is no direct report of biochar application reducing GHGs in aquaculture system, the meta-analysis of 296 observations in agriculture fields and laboratory incubation of different biochar shows a reduction of 5% CO_2, 20% N_2O, and 19% CH_4 after crop cycle (Song et al., 2016). The biochar reduces enzymatic activity of soil and the precipitation of CO_2 by binding with the biochar surface (Case *et al*., 2012). The enhanced soil aeration and reduced compaction effect of biochar inhibit denitrification and methanogenesis due to more oxygen being present. The reduction of CH_4 emission is also associated with decreased ratios of methanogenic archaea to methanotrophic proteobacteria, as the increase in oxygen supply due to biochar application supports a group of aerobic methanotrophs. Other than biochar, regulation of C:N ratio in pond sediment of intensified aquaculture system with the addition of carbohydrate source increases the growth of heterotrophic bacteria. It inhibits the growth of nitrifying bacteria, due to competition for nutrients (especially nitrogen) between heterotrophic and nitrifying bacteria, hence reduces N_2O emission from the aquaculture system (Hu *et al.,* 2014).

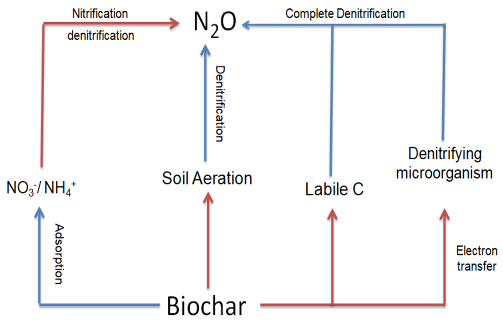


Fig.3. Biochar inhibits N_2O emission from soil.

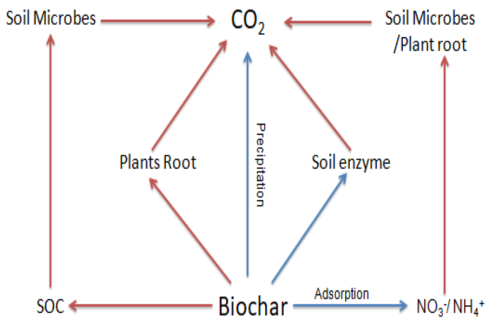


Fig.4. Biochar inhibits CO_2 emission from soil.

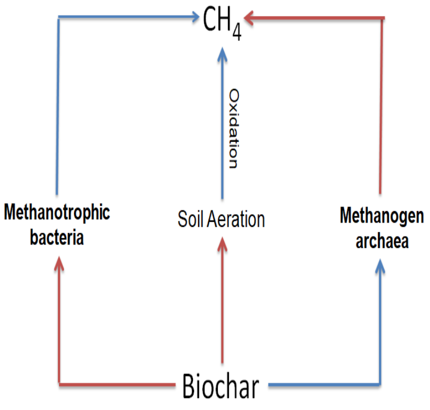


Fig.5. Biochar inhibits CH_4 emission from soil.

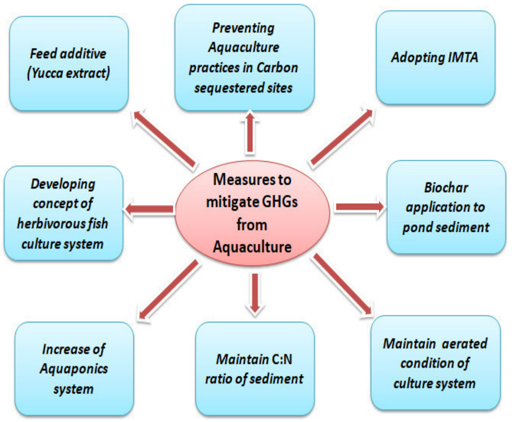


Fig.6. Mitigation strategies for ghgs emission from aquaculture system.

Conclusion

Protein demand from animal sources is increasing significantly with the increase in population. The recent supply of fish from aquaculture is running behind the demand of the global market. According to FAO, the demand for protein requirements from the aquaculture sector may increase up to 96% by 2050. The IFIF (International Feed Industry Federation) report reveals that currently livestock and fisheries consume 1 billion tons of formulated feed in the world which results in an indirect addition of 16 million tons of carbon into aquaculture systems. The emissions will increase with the growth in the aquaculture industry. The estimated damages by GHGs emission to eco-system and human health is about US$0.679 trillion and US$13 billion, respectively. There is a need for accurate and comprehensive estimation of GHGs emissions from different aquaculture systems and the different mechanisms of gas production so that the future strategic mitigation measure can be taken up for sustainable growth of the aquaculture sector.

References

- Adegbeye, M.J., M.M. Elghandour, J.C. Monroy, T.O. Abegunde, A.Z. Salem, A. Barbabosa-Pliego and T.O.Faniyi. 2019. Potential influence of Yucca extract as feed additive on greenhouse gases emission for a cleaner livestock and aquaculture farming-A review. Journal of Cleaner Production :118074.
- Case, S.D.C., N.P. Mcnamara, D.S Reay and J. Whitaker. 2012. The effect of biochar addition on N_2O and CO_2 emissions from a sandy loam soil – the role of soil aeration. Soil Biology and Biochemistry 51:125–134.
- Chen, Y., S. Dong, Z. Wang, F. Wang, Q. Gao, X. Tian and Y.Xiong. 2015. Variations in CO_2 fluxes from grass carp *Ctenopharyngodonidella* aquaculture polyculture ponds. Aquaculture Environment Interactions 8:31-40.
- http://cait.wri.org. date retrieved 8/1/2020
- https://ifif.org/global-feed/statistics/date retrieved 8/1/2020
- Hu, Z., J.W. Lee, K. Chandran, S.Kim and S. K. Khanal.2012.Nitrous oxide (N_2O) emission from aquaculture: a review.Environmental Science & Technology 46(12): 6470–6480.
- Hu, Z., J.W. Lee, K. Chandran, S. Kim, K. Sharma and Khanal. S.K., 2014. Influence of carbohydrate addition on nitrogen transformations and greenhouse gas emissions of intensive aquaculture system. Science of the Total Environment 470:193-200.
- Hu, Z., S. Wu, C. Ji, J. Zou, Q. Zhou and S.Liu. 2016. A comparison of methane emissions following rice paddies conversion to crab-fish farming wetlands in southeast China. Environmental Science and Pollution Research 23(2):1505-1515.
- IPCC (2014) Summary for policy markers. In: Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change (eds Stocker TF, D Qin, G-K Plattner, M Tignor, SK Allen, J Boschung, A Nauels, Y Xia, V Bex and Midgley PM), pp. 3–29. Cambridge University Press, Cambridge, UK and New York, NY, USA.

- Kauffman, J.B., A.F. Bernardino, T.O. Ferreira, N.W. Bolton, L.E.D.O. Gomes and G.N. Nobrega. 2018. Shrimp ponds lead to massive loss of soil carbon and greenhouse gas emissions in northeastern Brazilian mangroves. Ecology and evolution 8(11):5530-5540.
- Mao, H., J. Wang, Y. Zhou and J. Liu, 2010. Effects of addition of tea saponins and soybean oil on methane production, fermentation and microbial population in the rumen of growing lambs. Animal Feed Science and Technology. 129:56-62.
- Queiroz, H.M., A.G. Artur, C.A. Taniguchi, M.R. da Silveira, J.C. do Nascimento, G.N. Nóbrega, X.L. Otero, and T.O. Ferreira. 2019. Hidden contribution of shrimp farming effluents to greenhouse gas emissions from mangrove soils. Estuarine, Coastal and Shelf Science 221:8-14.
- Raul, C., S. Priyadarshi, V.S. Bharti and S. Prakash. 2019. Biochar: An Emerging Solution for Sustainable Aquaculture. World Aquaculture Society 50:64-65.
- Robb, D.H., M. MacLeod, M.R. Hasan and D. Soto. 2017. Greenhouse gas emissions from aquaculture: A life cycle assessment of three Asian systems. FAO Fisheries and Aquaculture Technical Paper, (609), Rome.
- Sidik, F. and C.E. Lovelock. 2013. CO2 efflux from shrimp ponds in Indonesia. PLoS One 8(6):66329.
- SOFIA. 2018. State of World fisheries and Aquaculture. FAO, Rome.
- Song, X., Pan, G., Zhang, C., Zhang, L. and H.Wang. 2016. Effects of biochar application on fluxes of three biogenic greenhouse gases: a meta-analysis. Ecosystem Health and Sustainability 2(2):1202
- Srinivasa R, C., M. Prabhakar, M. Maheswari, M. SrinivasaRao, K.L. Sharma, K. Srinivas, J.V.N.S. Prasad, C.A. Rama Rao, M. Vanaja, D.B.V. Ramana, and K.A. Gopinath. 2016. National Innovations in Climate Resilient Agriculture (NICRA), Research Highlights 2015-16. Central Research Institute for Dryland Agriculture, Hyderabad
- Yang, P., Q. He, J. Huang and C. Tong. 2015. Fluxes of greenhouse gases at two different aquaculture ponds in the coastal zone of southeastern China. Atmospheric Environment 115:269-277.
- Yang, P., Q. He, J. Huang, and C. Tong. 2015. Fluxes of greenhouse gases at two different aquaculture ponds in the coastal zone of south-eastern China. Atmospheric Environment 115:269-277.

9. Ammonia and Nitrate Removal from Wastewater by Using Biochar

Vidya Shree Bharti & Iffat Jahan
ICAR- Central Institute of Fisheries Education, Versova, Mumbai-400061

Ammonia removal is normally realized by sequentially alternating between oxic and anoxic conditions or by the creation of separated zones with suitable conditions for nitrification and denitrification, respectively. The removal of nitrogen from wastewater has become an emerging worldwide concern because it may cause eutrophication in natural water. Recently various agro-based biochar products biochar is found as effective in wastewater treatment. Nitrogen removal from waste water by using biochar might be cost-effective, less sludge production, efficient nutrient removal, high operational flexible and less operational complex process. Research to date shows that biochar potentially has the ability to manipulate the rates of N cycling in soil systems by influencing nitrification rates and adsorption of ammonia and increasing $NH4^+$ storage by enhancing cation exchange capacity in soils.

Principal

Water sample is treated in an alkaline citrate medium with sodium hypochlorite and phenol in the presence of sodium nitroprusside, which act as a catalyst. The blue indophenol colour formed with ammonia is measured spectrophotometrically.

Sample digestion

Add required biochar according to standardization (0.1g, 0.5 g etc.) in decided stocked solution of ammonium sulphate (e.g. 5 ppm). Mix it. Placed them on mechanical shaker for 1 hour for proper mixing. Thereafter, filtrate it by using filter paper. These sample now go for ammonia estimation.

Reagents

a) **De- ionised water**

b) **Sodium nitroprusside solution**: Dissolve 1g of sodium nitroprusside, in 200 ml of de-ionised water. Store in a dark glass bottle. The solution is stable for least a month.

c) **Phenol solution:** Dissolved 20g of analytical grade phenol in 200 ml of 95% ethyl alcohol.

d) **Alkaline reagent:** Dissolve 100g of sodium citrate and 5 g of sodium hydroxide in 500 ml of de-ionised water. The solution is stable indefinitely.

e) **Sodium hypochlorite solution:**

f) **Oxidising solution:** Mix 100 ml of reagent (d) and 25 ml reagent (e). Prepare fresh every day.

Procedure

Add 50 ml of seawater to an Erlenmeyer flask from 50 ml measuring cylinder. Add 2 ml of phenol solution, swirl to mix then add in 2 ml of nitroprusside 5 ml oxidising solution. Keep them for 1 hour by covering the flask. Read the absorbance at 640 nm in a spectrophotometer against blank or distilled water using 10 ml cell.

Calculation

Calculate the ammonia concentration by using calibrated curve.

Standard Curve

Dissolve 0.9433 g of analytical reagent quality ammonium sulphate in 950 ml of distilled water. This solution contains 200 ppm. Prepare a series of standard solutions from this stock solution and carry out the method exactly as describe above. After colour development, measure absorbance at 640 nm and prepare a calibration curve from the absorbance of series of standard.

Nitrate Removal

Principle:

The NO_3^- calibration curve follows Beer's law up to 11 mg N/L. Measurement of UV absorption at 220 nm enables rapid determination of NO3. Because dissolved organic matter also may absorb at 220 nm and NO3 does not absorb at 275 nm, a second measurement made at 275 nm may be used to correct the NO3 value. The extent of this empirical correction is related to the nature and concentration of organic matter and may vary from one water to another. Sample filtration is intended to remove possible interference from suspended particles. Acidification with 1N HCl is designed to prevent interference from hydroxide or carbonate concentrations up to 1000 mg CaCO3/L. Chloride has no effect on the determination.

Sample digestion:

Add required biochar according to standardization (0.1g, 0.5 g etc.) in decided stocked solution of Dry potassium nitrate (e.g. 1, 2, 5 ppm). Mix it. Placed them on mechanical shaker for 1 hour for proper mixing. Thereafter, filtrate it by using filter paper. These sample now go for nitrate estimation.

Reagents:

- **Nitrate-free water**: Use redistilled or distilled, deionized water of highest purity to prepare all solutions and dilutions.
- **Stock nitrate solution**: Dry potassium nitrate (KNO3) in an oven at 105°C for 24 h. Dissolve 0.7218 g in water. This solution is stable for at least 6 months.
- **Hydrochloric acid solution**: HCl, 1N.

Procedure:

- Treatment of sample: To 50 mL clear sample, filtered if necessary, add 1 mL HCl solution and mix thoroughly. b. Preparation of standard curve:

- Prepare NO_3^- calibration standards in the range 0 to 7 mg NO_3^--N/L by diluting to 50 mL the following volumes of intermediate nitrate solution: 0, 1.00, 2.00, 4.00, 7.00 . . . 35.0 ml.
- Treat NO_3^- standards in same manner as samples.

Spectrophotometric measurement:

- Read absorbance or transmittance against redistilled water set at zero absorbance or 100% transmittance. Use a wavelength of 220 nm to obtain $NO3^-$ reading and a wavelength of 275 nm to determine interference due to dissolved organic matter.

Calculation

- For samples and standards, subtract two times the absorbance reading at 275 nm from the reading at 220 nm to obtain absorbance due to $NO3^-$. Construct a standard curve by plotting absorbance due to $NO3^-$ against $NO3^-$-N concentration of standard. Using corrected sample absorbances, obtain sample concentrations directly from standard curve.

10. Carbon Sequestration in Aquatic Environment: Role of Algae

Satya P. Shukla & Ashutosh Kumar Singh
ICAR- Central Institute of Fisheries Education, Versova, Mumbai-400061

Introduction

Algae are gaining popularity as one of the most efficient biological system for the production of biomass through photosynthesis. The algae sequester a significant amount of atmospheric carbon through carbon fixation during photosynthesis and therefore, reduce the concentration of carbon dioxide in the atmosphere. Therefore, atmospheric carbon sequestration through algae is an effective tool for mitigation of global warming. During recent past, atmospheric carbon sequestration through algae biomass production is emerging as a promising strategy for global warming mitigation (Shukla et al., 2017). An added advantage of algae based carbon sequestration is the recovery of value-added compounds derived from algae through down-stream processing. These compounds have a wide range of potential applications in aquaculture, human nutrition and health products (Satapathy et al, 2015; Shukla et al., 2013; Sandeep et al , 2015; Shukla et al , 2008). Recently, microalgae have emerged as a rich source of lipids which can be converted to biodiesel through transesterification.

Advantages of Algae Biomass Production

Algae require minimum quantity of water and chemicals for their growth and the biomass can be produced and harvested daily except for few days of rainy months. The cost of production can be further minimized by using industrial or municipal waste water and sea water.

There is no conflict with food crops for water or land, therefore, food security is not at the stake in case of algal biodiesel production.

Microalgae are considered to be suitable candidates for atmospheric carbon sequestration by virtue of attributes such as faster growth, ability to grow in low quality water, and tolerance towards a wider range of temperature, salinity and nutrient deficient environment. Further, the downstream processing of micro-algal biomass yields a variety of value added products including biodiesel which is considered to be a lucrative alternative to fossil based fuels.

An unprecedented increase in atmospheric carbon dioxide during post-industrial era has posed a serious challenge to the global ecosystems and their sustainability (Stewart and Hessami, 2005; Kumar et al, 2010). An appreciable number of research and development initiatives were taken during recent past directing the efforts for reducing the atmospheric carbon dioxide through innovative technologies (Tsai et al, 2015). Among the mitigation strategies, the prominent ones are:

I. Chemical reaction based approaches such as washing with alkaline solution (Diao et al, 2004), multi walled carbon nanotubes (Sui et al., 2009) and amine coated activated carbon (Plaza et al, 2007).
II. Direct injection to underground (Herzog et al, 2001) or to the ocean (Israelsson et al., 2009).
III. Biological carbon dioxide fixation through photosynthetic microorganisms, algae and terrestrial plants (Skjaner et al., 2007).

Among the carbon fixing organisms, microalgae and cyanobacteria have few advantages such as faster growth as compared to terrestrial plants and 10-50 times higher carbon dioxide fixation efficiency (Costa et al , 2000). There is a considerable interest worldwide in microalgae based carbon dioxide sequestration because the biomass of microalgae and cyanobacteria could be used for wide ranging valuable products such as biofuels, medications, cosmetics and nutraceuticals (de Morais and Costa, 2007). Apart from the precursors of value added products, micro-algal carbon dioxide fixation is environmentally sustainable as it can be combined with various environment friendly processes such as wastewater treatment (Mallik, 2002) and heavy metal removal (Jacone – Pilco et al., 2009).

Algal Biochar

Algal biochar has been recognized for its potential for carbon sequestration and soil amelioration. Application of biochar improves the water holding capacity of soil and provides a substrate for enhanced microbial activity (Thies and Rillig, 2009). Algae cab be cultured on nonarable land and can utilize water of varying salinity and nutrient regimes. Therefore, the biomass of algae has become an attractive feed stock for biochar production. Macroalgae show comparatively lower carbon content compared to many terrestrial plants, however, the nitrogen , phosphorus , potassium and other nutrients are in higher quantity in algal biochar. One of the potential area for the application of algal biochar is integrated aquaculture-agriculture system where the nutrient rich effluents from aquaculture-agriculture production are used for algae biomass production. The algal biochar production offers multiple benefits including the value added products (pigments, lipids, protein etc) from the biomass before the conversion of the residual biomass to biochar.

Suggested Readings

- Acien-Fernandez FG, Fernandez Sevilla JM, Sanchez Perez JA, Molina Grima E, Chisti Y (2001) Airlift-driven external loop tubular photobioreactors for outdoor production of microalgae: assessment of design and performance. Chem Eng Sci 56:2721-2732
- Arjun RK, Lipin D, Thankamani V (2012) An integrated process for industrial effluent treatment and biodiesel production using microalgae. Res Biotechnol 3(1):47-60
- Boussiba S, Sandbank E, Shelef G, Cohen Z, Vonshak A, Ben-Amotz A, Arad S, Richmond A(1988) Outdoor cultivation of the marine microalgae Isochrysis galbana in open reactors. Aquacult 72:247-253
- Camacho Rubio F, Acien Fernandez FG, Sanchez Perez JA, Garcia Camacho F, Molina Grima E (1999) Prediction of dissolved oxygen and carbon dioxide concentration profiles in tubular photobioreactors for microalgal cultures. Biotechnol Bioenergy 62:71-86
- Carlozzi P (2003) Dilution of solar radiation through culture lamination in photobioreactor rows facing south-north: a way to improve the efficiency of light utilization by cyanobacterium (*Arthrospira platensis*). Biotechnol Bioenerg 81:305-315
- Carvalho AP, Malcata FX (2001) Transfer of carbon dioxide within cultures of microalgae: plain bubbling versus hollow fiber modules. Biotechnol Prog 17:265-272
- Carvalho AP, Meireles LA, Malcata FX (2006) Microalgal reactors: a review of enclosed system designs and performances Biotechnol Prog 22:1490-1506
- Cheng-Wu Z, Zmora O, Kopel R, Richmond A (2001) An industrial size flat glass reactor for mass production of *Nannochloropsis sp* (Eustigmatophyceae). Aquacult 195:35-49

- Costa JAV, Linde GA, Atala DIP (2000) Modelling of growth conditions for cyanobacterium *Spirulina platensis* in microcosms. World J Microbiol Biotechnol 16:15-18
- De Morais MG, Costa JAV (2007) Biofixation of carbon dioxide by Spirulina sp and Scenedesmus obliquus cultivated in a three-stage serial tubular photobioreactor. J Biotechnol 129: 439-445
- Diao YF, Zheng XY, He BS, Chen CH, Xu XC (2004) Experimental study on capturing CO_2 greenhouse gas by ammonia scrubbing. Energy Consr Managmt 45:2283-2296
- Garcia-Malea Lopez MC, Del Rio Sanchez E, Casas Lopez JL, Acien Fernandez FG, Fernandez Sevilla JM, Rivas J, Guerrero MG, Molina Grima (2006) Comparative analysis of the outdoor culture of Heamatococcus pluvialis in tubular and bubble column photobioreactors. J Biotechnol 123:329-342
- Grierson S, Strezov V, Herbertson J, Ellem G, Mc Gregor R (2009) Thermal characterization of microalgae under slow pyrolysis conditions. J Analytic Appl Pyrolysis 85:118-123
- Hall DO, Fernandez FGA, Gerrero EC, Rao KK, Grima EM (2003) Outdoor helical tubular photobioreactors for microalgal production; modeling of fluid ynamics and mass transfer and assessment of biomass productivity. Biotechnol Bioeng 82:62-73
- Herzog H (2001) What future for carbon capture and sequestration? Environ Sci Technol 35:148A-153A
- Hoekema S, Bijmans M, Janssen M, Tramper J, Wijffels RH (2002) A pneumatically agitated flat-panel photobioreactor with gas recirculation; anaerobic photoheterotrophic cultivation of purple non-sulfur bacterium. Int J Hydro Energ 27:1331-1338
- Israelsson PH,Chow AC,Adam EE(2009) An updated assessment of the cute impacts of ocean carbon sequestration by direct injection. Int J Greenhouse Gas Cont 4:262-271
- Jacob-Lopes E, Scoparo CHG, Lacerda L, France TT(2009) Effect of light cycles(night/day) on CO_2 fixation and biomass production by microalgae in photobioreactor. Chem Eng Proc 48:306-310

- Jacome Pilco CR, Cristiani-Urbania E, Flores-Cotera LB, Velasco-Garcia R, Ponce-Noyola T, Canizares-Villanueva RO(2009) Continuous Cr(VI) removal by *Scenedesmus incrassulatus* in an airlift photobiorecator. Bioresource Technol 100:2388-2391
- Kaewpintong K, Shotipruk A, Powtongsook S, Pavasant P(2007) Photoautotrophic high-density cultivation of vegetative cells of Haematococcus pluvialis in airlift bioreactor. Biores Technol 98:288-295
- Kumar A , Sarina Ergas , Yuan Xin, Sahu A, Zhang Q, Dewalf J, Malcata X, van Langenhove H(2010) Enhanced CO_2 fixation and biofuel production via microalgae : recent developments and future directions. Trends in Biotech 28:317-380
- Lee JS, Kim DK, Lee JP,Park SC, Koh JH, Cho HS (2002) Effects of SO_2 and NO on growth of Chlorella sp KR-1 Biores Technol 82:1-4
- Lee YK, Low CS (1991) Effects of photobioreactor inclination on the biomass productivity of an outdoor algal culture. Biotechnol Bioeng 38:995-1000
- Lehmann J, Joseph S (2009) Biochar for environmental management. Earthscan, Virginia ISBN 978-1-84407-658-1
- Maeda K, Owada M, Kirmura N, Omata K, Karube I (1995) CO_2 fixation from the flue gas on coal fired thermal power plant by microalgae. Energy Conserv Manage 36:717-720
- Mallik N (2002) Biotechnological potential of immobilized algae for wastewater N,P and metal removal: a review. Biometals 15:377-390
- Matsunaga T, Takeyama H, Sudo H, Oyama N, Ariura S, Takano H, Hirana M, Burgess JG, Sode K, Nakamura N(1991) Glutamate production from CO_2 by marine cyanobacterium *Synechococcus sp* using a novel biosolar reactor employing light diffusing optical fibers. Appl Biochem Biotechnol 28/29:157-167
- Milner HW (1953) Rocking tray In: Burlew JS(Ed) Algal cultures from laboratory to pilot plant. Carnegie Institution, Wasington DC p108 No 600
- Molina E, Fernandez J, Acien FG, Chisti Y (2001) Tubular photobioreactor design for algal cultures. J Biotechnol 92:113-131

- Mussugnug JH, Thomas-Hill S, Rupprecht J, Foo A, Klessen V, Mc Dowell A (2007) Engineering photosynthetic light capture impacts on improved solar energy to biomass conversion. Plant Biotechnol 5: 802-814
- Ogbonna JC, Soejima T, Tanaka H (1999) An integrated solar and artificial light system for internal illumination of photobioreactors J Biotechnol 70:289-297
- Olaizola M (2000) Commercial production of astaxanthin from *Haematococcus pluvialis* using 25000liter outdoor photobioreactors. J Appl Phycol 12:499-506
- Ono E, Cuello JL (2007) Carbon dioxide mitigation using thermophilic cyanobacteria Biosystem Bioeng 96:129-134
- Ono E, Cuello JL (2007) Carbon dioxide mitigation using thrmophilic cyanobacteria Biosys Bioeng 96:129-134
- Ota M, Kato Y, Watanabe H, Watanabe M, Sato Y, Smith RL(2009) Fatty acid production from a highly CO_2 tolerant alga Chlorococcum littorale in the presence of inorganic carbon and nitrate. Biores Technol 100:5237-5248
- Plaza MG, Pevida C, Arenillas A, Rubiera F, Pis JJ (2007) CO_2 capture by adsorption with nitrogen enriched carbon. Fuel 86:2204-2012
- Pulz O (2001) Photobiorectors: production systems for phototrophic microorganisms. Appl Env Biotechnol 57:287-293
- Pulz O, Gross W (2004) Valuable products from biotechnology of microalgae. Appl Microbiol Biotechnol 65:635-648
- Richmond A (2000) Microalgal biotechnology at the turn of the millennium; a personal view J Appl Phycol 12:441-451
- Richmond A, Boussiba S, Vonshak A, Kopel R (1993) A new tubular reactor for mass production of microalgae outdoors. J Appl Phycol 5:327-332
- Ross A, Jones JM, Kubacki ML, Bridgeman TG(2008) Classification of macroalgae as fuel and its thermochemical behavior. Biores Technol 99:6494-6504
- Sakai N, Sakamoto Y, Kishimoto N, Chihara M, Korube I (1995) Chlorella strains from hot springs tolerant to high-temperature and high CO_2 . Energ Conserv Manag 36:693-696

- Sanchez-Miron A, Ceron Garcia MC, Garcia Camacho F, Molina Grima E, Chisti Y(2002) Growth and characterization of microalgal biomass produced in bubble column and airlift photobiorecators; studies in fed-batch cultures Enzyme Microbiol Technol 31:1015-1023
- Shukla SP, Kviderova J, Triska J, Elster J(2013) *Chlorella mirabilis* as a potential species for biomass production in low-temperature environment. Frontiers in Microbiology Doi 10.3389/fmicb.2013.00097
- Shukla SP, Mishra AK, Kashyap AK (1997) Influence of low temperature and salinity stress on growth behaviour and pigment composition of Antarctic and tropical isolates of a diazotrophic cyanobacterium *Anabaena* Ind J Exp Biol 35(11):1224-1228
- Skjanes K, Lindblad P, Muller J(2007) Bio CO_2 – a multidisciplinary , biological approach using solar energy to capture CO_2 while producing H_2 and high value products. Biomol Eng 24:405-413
- Stewart C, Hessami MA (2005) A study of methods of carbon dioxide capture and sequestration- the sustainability of photosynthetic bioreactor approach. Energy Conserv Managmt 46:403-420
- Su FS, Lu CS, Cnen WF, Bai HI, Hwang JF (2009) Capture of CO_2 from flue gas via multiwalled carbon nanaotubes. Sci Total Environ 407:3017-3023
- Sung KD, Lee JS,Shin CS, Park SC (1999) Isolation of a new highly CO_2 tolerant freshwater microalgae Chlorella sp KR-1.Renew Energ 16:1019-1022
- Thies JE, Rilliz MC(2009) Characteristics of biochar: biological properties In: Lehmann S, Joseph J(Eds) Biochar for Environmental Management, Earthscan, Virginia pp85-106
- Torzillo G, Pushparaj B, Bocci F, Balloni W, Materassi R, Florenzano G(1986) Production of Spirulina biomass in closed photobioreactors. Biomass 11:61-74
- Tredici MR (2010) Photobiology of microalgae mass culture; understanding the tools for the next green revolution. Biofuels 1: 143-162
- Tredici MR, Chini Zitelli G (1998) Efficiency of sunlight utilization; tubular versus flat photobioreactors. Biotechnol Bioeng 57:187-197

- Tredici MR, Materassi R(1992) From open ponds to vertical alveolar ponds: the Italian experience in the development of reactors for the mass cultivation of photoautotrophic microorganisms J Appl Phycol 4:221-231
- Tsai DDW, Chen PH, Chou CMJ, Hsu CF, Ramraj R (2015) Carbon sequestrationby algae ecosystem. Ecol Eng 84:386-389
- Ugwu CU, Aoyagi H, Uchiyama H (2008) Photobioreactors for mass cultivation of algae. Biores Technol 99:4021-4028
- Ugwu CU, Ogbonna JC, Tanaka H (2002) Improvement of mass transfer characteristics and productivities of inclined tubular photobioreactors by installation of internal static mixers Appl Microbiol Biotechnol 58:600-607
- Ugwu CU, Ogbonna JC, Tanaka H (2003) Design of static mixers for inclined tubular photobiorectors. J Appl Phycol 15:217-223
- Xu L, Weathers PJ, Xiong XR,Liu CZ (2009) Microalgal bioreactors: challenges and opportunities. Eng Life Sci 9:178-189
- Yang Y, Gao K(2003) Effects of CO_2 concentration on the freshwater microalgae Chlamydomonas reinhardtii, Chlorella pyrenoidosa and Scenedesmus obliquus (Chlorophyta) J Appl Phycol 15:379-389
- Yue LH, Chen WG (2005) Isolation and determination of cultural characterization of a new highly CO_2 tolerant freshwater microalgae. Energy Conserv Managmt 46:1868-1876
- Zhang K, Kurano N, Miyachi S (2002) Optimized aeration by carbon dioxide gas for micro-algal production and mass transfer characterization in a vertical flat-plate photobioreactor. Bioprocess Biosyst Eng 25:97-101

11. Concepts of Bioremediation and its Application with Biochar

Rathi Bhuvaneswari G.
ICAR- Central Institute of Fisheries Education, Versova, Mumbai-400061

Introduction

Intensification of agriculture and industrialisation has resulted in increased release of a wide range of xenobiotic compounds, which have put on increasing burden on the environment and also have inflicted serious damage on the ecosystem. The conventional techniques used for remediation of those pollutants have been to dig up contaminated soil and remove it to a landfill, or to cap and contain the contaminated areas of a site. A better approach than these traditional methods is to completely destroy the pollutants if possible, or at least to transform them to innocuous substances. Some technologies that have been used are incineration and various types of chemical oxidation. The former method leads to gaseous phase pollution through obnoxious gas release and the later would be expensive and also generates a large quantum of waste. Hence a sustainable and ecofriendly alternative to the above said menace would be bioremediation which offers the possibility to destroy or render harmless various contaminants using natural biological activity.

Bioremediation is defined as the process by which biological agents – microorganisms or their products are stimulated to rapidly degrade hazardous organic pollutants to environmentally safe levels in water, soil, sediments and other substances. Bioremediation technology uses microorganisms to reduce, eliminate, contain, or transform to benign contaminants present in soils, sediments, water, and air. Recently, biological remediation process have also been devised to either precipitate, effectively immobilize, inorganic pollutants such as heavy metals. Stimulation of microorganisms is achieved by the addition of growth substances, nutrients, terminal electron acceptor or donors or some combination thereby resulting in an increase in organic pollutant degradation and bio-transformation.

Principle of Bioremediation

There are three essential components needed for bioremediation. These three components are microorganisms, food, and nutrients. These three main components are known as the bioremediation triangle

The contaminant serves two useful purposes for the microbes.

- First, the contaminant provides a source of carbon needed for growth.
- Second, the microbes obtain energy by breaking chemical bonds and transferring electrons away from the contaminant. This is known as an oxidation reduction reaction.

The contaminant that loses electrons is oxidized and the chemical that gains the electrons (electron acceptor) is reduced. The energy gained from the electron transfer is used along with the carbon and some electrons to produce more cells. Microbes generally use oxygen as an electron acceptor but nitrate, sulfate, iron (III)- and CO_2 are also commonly used. Redox potentials provide an indication of the relative dominance of the electron acceptor classes. Most bioremediation systems are run under aerobic conditions, but running a system under anaerobic conditions may permit microbial organisms to degrade otherwise recalcitrant molecules

Factors affecting bioremediation:

The following are the key factors which affect the bioremediation process

Contaminant concentrations: Directly influence microbial activity. When concentrations are too high, the contaminants may have toxic effects on the present bacteria. In contrast, low contaminant concentration may prevent induction of bacterial degradation enzymes.

Contaminant bioavailability: Depends on the degree to which they sorb to solids or are sequestered by molecules in contaminated media, are diffused in macropores of soil or sediment. Bioavailability for microbial reactions is lower for contaminants that are more strongly sorbed to solids, enclosed in matrices of molecules in contaminated media, more widely diffused in macropores of soil and sediments.

Site characteristics: Have a significant impact on the effectiveness of any bioremediation strategy. Site environmental conditions important to consider for bioremediation applications include pH with an optimum in the range of 6-8, temperature, water content, nutrient availability,

Redox Potential and oxygen content: typify oxidizing or reducing conditions. Redox potential is influenced by the presence of electron acceptors such as nitrate, manganese oxides, iron oxides and sulphate.

Nutrients: Are needed for microbial cell growth and division. Suitable amounts of trace nutrients for microbial growth are usually present, but nutrients can be added in a useable form or via an organic substrate amendment (Parsons 2004), which also serves as an electron donor, to stimulate bioremediation.

Moisture content: Microbial growth requires an optimum presence of water in the environmental matrix. For optimum growth and proliferation, microorganisms require 12% to 25% of moisture.

Temperature: Directly affects the rate of microbial metabolism and consequently microbial activity in the environment. The biodegradation rate, to an extent rises with increasing temperature and slows with decreasing temperature (ESTCP 2005).

Bioremediators

There are some groups of microbes which are being used in bioremediation such as;

Aerobic: Aerobic bacteria recognized for their degradative abilities are *Pseudomonas* sp, *Sphingomonas sp, Rhodococcus sp, Mycobacterium sp, Serratia sp, Bacillus sp.* These microbes have often been reported to degrade pesticides and hydrocarbons, both alkanes and polyaromatic compounds. Many of these bacteria use the contaminant as the sole source of carbon and energy.

Anaerobic: Anaerobic bacteria are not as frequently used as aerobic bacteria. There is an increasing interest in aerobic bacteria used for bioremediation of polychlorinated biphenyls in river sediments, dechlorination of the solvent trichloroethylene and chloroform. *Dechloromonas aromatica – Benzene, Paracoccus denitrificans* & *Thiobacillus s*p– Nitrate and ammonium.

Mechanisms of Heavy Metal Uptake by Microorganisms

The cellular structure of a microorganism can trap heavy metal ions and subsequently sorb them onto the binding sites of the cell wall. This process is called biosorption or passive uptake, and is independent of the metabolic cycle.

The peptidoglycan layer in Gram-positive bacteria, which contains alanine, glutamic acid, meso-di-aminopimelic acid, polymer of glycerol and teichoic acid, and that of the Gram-negative bacteria, which contains enzymes, glycoproteins, lipopolysaccharides, lipoproteins, and phospholipids, are the active sites involved in metal binding processes

The other method is a process in which the heavy metal ions pass across the cell membrane into the cytoplasm, through the cell metabolic cycle. This is referred to as bioaccumulation or active uptake.

Enzymes:

The process of bioremediation is a very slow process. Only certain species of bacteria fungi and microalgae have proven their ability as potent pollutant degraders. Many strains are known to be effective as bioremediation agents but only under laboratory conditions. The limitation of bacterial growth is under the influence of pH, temperature, oxygen, soil structure, moisture and appropriate level of nutrients, poor bioavailability of contaminants, and presence of other toxic compounds. Although microorganisms can exist in extreme environment, most of them prefer optimal condition a situation that is difficult to achieve outside the laboratory. Most bioremediation systems operate under aerobic conditions, but anaerobic environments may also permit microbial degradation of recalcitrant molecules. Both bacteria and fungi rely on the participation of different intracellular and extracellular enzymes respectively for the remediation of recalcitrant and lignin and organopollutants.

All known enzymes fall into one of these six categories. The six main divisions are

- Oxidoreductases
- Transferases
- Hydrolases
- Lyases
- Isomerases
- Ligases (synthetases)

Oxidoreductases catalyze the transfer electrons and protons from a donor to an acceptor.
Transferases catalyze the transfer of a functional group from a donor to an acceptor.
Hydrolases facilitate the cleavage of C–C, C–O, C–N, and other bonds by water.
Lyases catalyze the cleavage of these same bonds by elimination, leaving double bonds
Isomerases - facilitate geometric or structural rearrangements or isomerizations.
Ligases catalyze the joining of two molecules

Types of Bioremediation:

On the basis of removal of wastes for treatment there are basically two methods:-
1. In situ bioremediation
2. Ex situ bioremediation

In situ bioremediation

The bioremediation process is carried onsite. In situ bioremediation technology was originally developed as a less costly, more effective alternative to the standard pump-and-treat methods used to clean up aquifers and soils contaminated with organic chemicals(e.g., fuel hydrocarbons, chlorinated solvents), but has since expanded in breadth to address explosives, inorganics (e.g. nitrates), and toxic metals (e.g., chromium)

- Applied to soil & ground water at the site
- Direct contact between microbe and contaminant
- Minimal site disturbance
- Simultaneous treatment of soil and ground water
- Minimal exposure of public
- Low cost

Chemotaxis is important to the study of in situ bioremediation because microbial organisms with chemotactic abilities can moves into an area containing contaminants. So by enhancing the cells chemotactic abilities, in-situ bioremediation will become a safer method in degrading harmful compounds. In-situ bioremediation is classified into to types Intinsic and Engineered bioremediation

Intrinsic bioremediation

Conversion of environmental pollutants into the harmless forms through the innate capabilities of natural occurring microbial population

Engineered in situ bioremediation

Construction of engineered systems to supply materials that stimulate microbes by the following methods

Biostimulation: It provides nutrients, electron donor and suitable physiological conditions for the growth of the indigenous microbial populations

Bioaugmentation: Addition of microbes indigenous or exogenous or their enzyme to the contaminated sites. It enhances transformation or stabilization of specific pollutants

Bioventing: supplying air through wells to stimulate the indigenous bacteria. Mainly for hydrocarbons and less volatile substance also where contamination is deep

Biosparging: Injection of air under pressure below the water table to increase oxygen concentration. It is effective in treating chlorinated solvents contamination

Ex situ bioremediation: Bioremediation carried on off site

It involves removal of waste & their collection at a place to facilitate microbial degradation. High cost is associated with excavation, screening, mixing, homogenisation & disposal. On basis of phases ex- situ remediation is divided into

- Solid phase system
- Slurry phase system

Land farming: Soil is excavated and spread over a prepared bed and periodically tilled until pollutants are degraded by stimulating indigenous microbes.Relatively shallow layers of soil can be treated **and** less monitoring and maintenance cost is involved

Composting: Involves combining contaminated soil with nonhazardous organic substances such as manure or agricultural wastes. The organic materials support development of a rich microbial population mainly used for bioremediation of aliphatic & aromatic & certain halogenated compounds.

Bioreactors: It has been developed to achieve better control over the environmental conditions. Controlling the factors such as temperature, pH, oxygen concentrations, nutrient input help accelerate biodegradation process.

Bioremediation in aquaculture

The wastes in aquaculture farms can be categorized as residual food, metabolic by product, residues of biocides, fertilizer derived wastes and wastes produced during moulting and collapsing algal blooms. Beneficial bacteria produce a variety of enzymes that break down the said waste of aquaculture including proteins and starch to small molecules, which are then taken up as energy sources by other organisms. Members of the genus *Bacillus,* like *Bacillus subtilis, Bacillus licheniformis*, *Bacillus cereus*, *Bacillus coagulans,* and of the genus *Paenibacillus*, like *Paenibacillus polymyxa,* are good examples of bacteria suitable for bioremediation of organic detritus. *Lactobacillus* is also used along with *Bacillus* to break down the organic detritus. The removal of large organic compounds reduces water turbidity. For the removal of ammonia from closed aquaculture systems, bacteriological nitrification is the most practical method and it is commonly achieved by setting of sand and gravel bio-filter through which water is allowed to circulate. The ammonia oxidizers are placed under five genera, *Nitrosomonas, Nitrosovibrio, Nitrosococcus, Nitrolobus* and *Nitrospira,* and nitrite oxidizers under three genera *Nitrobacter, Nitrococcus* and *Nitrospira* The vast majority of aquaculture ponds accumulate nitrate, as they do not contain a denitrifying filter. Denitrifying filters helps to convert nitrate to nitrogen. It creates an anaerobic region where anaerobic bacteria can grow and reduce nitrate to nitrogen gas (Rao, 2002). Among these, *Pseudomonas, Bacillus* and *Alcaligenes* are the most prominent numerically. Unionized H2S is extremely toxic to fish at concentrations that may occur in natural waters as well as in aquaculture farms. Under anaerobic conditions the photosynthetic benthic bacteria that break H2S at pond bottom have been widely used in aquaculture to maintain a favourable environment. These bacteria contain bacteria chlorophyll that absorbs light (blue to infrared spectrum, depending on type of bacterio-chlorophyll) and performs photosynthesis under anaerobic conditions. They are purple and green sulphur bacteria that grow at the anaerobic portion of the sediment-water interface

Biochar in Bioremediation

Biochar is porous, carbon-rich, and solid material produced by the thermochemical conversion of waste biomass under anoxic or anaerobic conditions. Waste biomass, used widely in biochar production, includes: crop residues, forestry waste, animal manure, food processing waste, paper mill waste, municipal solid waste, and sewage sludge Owing to its large surface area, developed pore structure, and richness of functional groups, biochar could co-adsorb organic pollutants along with native organic matter in the soil. When applied to the environment, biochar reduces the bioavailability of contaminants associated with accumulation and toxicity in plants and animals, with additional benefits of soil fertilization and mitigation of climate change. The sorption capacity of biochar depends highly on parameters such as pyrolysis conditions, including residence time, feedstock types, temperature, and heat transfer rate. Similarly, the physicochemical properties such as surface area, surface charge, and chemical functionality are influential factors controlling the sorption of inorganic and organic contaminants. Their high adsorption capacities, environmental viability, and cost effectiveness have demonstrated that biochars obtained from waste biomass have high potential for removing heavy metals and organic contaminants such as synthetic dyes, antibiotics, PAHs, and organic pesticides from different environments. Adsorption mechanisms and capacities depend on the nature of the pollutants and preparation conditions for the biochars. Thus selectivity of suitable biochar is crucial and needs more attention. In immobilised form associated with bacteria, it was found hat biochar provides shelter and nutrition to bacteria, resists or improves lower pH, reduces toxicity to bacteria and finally higher degradation of the pollutant.

Conclusion

Bioremediation is a powerful tool available to clean up contaminated sites. The idea of bioremediation has a long history. However, other applications are relatively new and many other applications are emerging or being developed. Bioremediation occurs when the microorganisms can biodegrade the given contaminant and the necessary nutrients such as nitrogen, phosphorus, electron acceptors, and trace elements. This process can be aerobic or anaerobic depending on the microorganisms and the electron acceptors available. This process may be natural (intrinsic bioremediation) or it may be enhanced by man (engineered bioremediation). Regardless of which aspect of bioremediation that is used; this technology offers an efficient and cost effective way to treat contaminated ground water and soil and hence very well suitable for aquaculture. The use of biochar immobilized with other bioremediators further increases the degradation efficiency which paves way for higher scope in pollutant remediation.

Suggested Readings

- Vidali, M., 2001. Bioremediation. an overview. *Pure and applied chemistry*, *73*(7), pp.1163-1172.
- Chávez-Crooker, P. and Obreque-Contreras, J., 2010. Bioremediation of aquaculture wastes. *Current Opinion in Biotechnology*, *21*(3), pp.313-317.
- Panigrahi, A., Mohapatra, A. and Panigrahi, A., 2005. Bioremediation An Environmental Friendly Approach For sustainable Aquaculture.
- Chen, B., Yuan, M. and Qian, L., 2012. Enhanced bioremediation of PAH-contaminated soil by immobilized bacteria with plant residue and biochar as carriers. *Journal of Soils and Sediments*, *12*(9), pp.1350-1359.
- Lyu, H., Gong, Y., Gurav, R. and Tang, J., 2016. Potential application of biochar for bioremediation of contaminated systems. In *Biochar Application* (pp. 221-246). Elsevier.

12. Potential Applications of Biochar in Fish Health Management

Gayatri Tripathi & K. Dhamotharan

ICAR- Central Institute of Fisheries Education, Versova, Mumbai-400061

Introduction:

In India, about 435.98 million tons of agro-residues are produced every year, out of which 313.62 million tons are surplus. These residues are either partially utilized or un-utilized due to various constraints. Biochar is one such component which helps in utilization of the such biomass. Biochar is a product of an exothermic pyrolysis process from plant biomass waste and has high organic carbon, which is stable in soil for a long time due to its recalcitrant property. It is pyrogenic carbonaceous material (PCM) produced by thermochemical conversion. Biochar undergoes activation process when processed with water vapour or CO_2 at temperatures above 750°C or chemical compounds like phosphoric acid and potassium chloride, which is known as activated biochar or activated carbon. When produced from pure stem wood, the solid phase of the pyrogenic process is known as charcoal. In contrast, the term biochar indicates that a broad spectrum of biogenic materials can serve as feedstock. Biochar activated carbon and charcoal can all be considered as pyrogenic carbon materials. In agriculture and animal husbandry biochar is utilized for improving water and soil quality, productivity and as feed supplements. Biochar as a climate-resilient product can be used for carbon sequestration to remove greenhouse gases such as carbon dioxide (CO_2), methane, and nitrous oxide [1]. Biochar is a hygroscopic material that can retain the nutrients and improve soil fertility. In agriculture, the biochar application has induced and improved plant responses to fungal diseases [2]. Biochar is rich in potassium. Biochar is one of the prominent candidate for the soil amendment because of its highly porous structure which is potentially responsible for increased water retention and surface area of soi. Feedstock type (agricultural residue, wood, animal waste, and aquatic waste), as well as pyrolysis temperature, can significantly influence biochar properties and hence its adsorption capacity. The physical and chemical characteristics of the biochar make it the right candidate for the removal of organic, inorganic, heavy metal pollutant, and wastewater effluent treatment for environmental remediation. Since biochar-carbon decomposes much slower than the original biomass, the application and use of biochar is considered as a terrestrial carbon sink on at least a centennial scale and is therefore a promising negative emission technology.

National and International Scenario of Biochar use in Animal Husbandry:

India produces tons of crops every year which makes it natural that a huge volume of vrop residues are produced from both on-farm and off-farm. It is estimated that approximately 500-550 Mt of crops residues are produced per year in the country. Most wastes are either burnt or end up in landfill, which degrade the environment and also produce large amounts of GHGs. The production of biochar from farm wastes and their application in farm soils offer multiple environmental and financial benefits. Biochar use has a very promising potential for the development of sustainable agricultural systems in India, and also for global climate change mitigation. It has been reported that use of biochar contributes in the climate change mitigation by increasing the plant productivity and crop yield which leads to the reduction og green house gases (Parmar *et al*, 2014). Biochar is a good source of basic cations such as calcium, magnesium, and potassium. Characterization studies have revealed that biochar has high content of potassium and nitrogen (Bharti *et al.,* 2018). Galvano *et al.* ,1996 have showed that the aflatoxin concentration in feed declined by 41 to 74%, while aflatoxin in milk was reduced by 22 to 45% during the week the cows received activated carbon in their diet. Toxins such as dioxin, glyphosate, mycotoxins, pesticides and PAHs are efficiently bound by the biochar. Feeding biochar can stimulate the activity of desired microorganisms like lactobacilli, enterococci and some yeasts in the digestive system (Gerlach *et al.,* 2018). It has been proposed by Schmidt *et a.l,* 2017 that If the global livestock would receive 1% of their feed in form of such a biochar, a total of about 400 Mt of CO_2eq or 1.2 % of the global CO_2 emissions could be compensated.

Biochar Production:

Biochar is a charcoal-like substance that's made by burning organic material from agricultural and forestry wastes (also called biomass) in a controlled process called pyrolysis. Pyrolysis is the most common technique employed to produce biochar, and also occurs in the early stages of the combustion and gasification processes. Besides biochar, bio-oil and gas can be collected from modern pyrolysers. During pyrolysis organic materials, such as wood chips, leaf litter or dead plants, are burned in a container with very little oxygen. As the materials burn, they release little to no contaminating fumes. During the pyrolysis process, the organic material is converted into biochar, a stable form of carbon that can't easily escape into the atmosphere. The energy or heat created during pyrolysis can be captured and used as a form of clean energy.

It is important to note that there is a wide variety of char products produced industrially such as activated carbon, char may be produced at high temperature, under long heating times and with controlled supply of oxygen. Carbonization is traditional charcoal production method, which involves smothering of biomass with soil prior to ignition or combustion of biomass while torrefaction is drying and roasting of biomass at even lower temperatures. Biochar from pyrolysis, and conventional charcoal and char share key characteristics which are related to carbon sequestration (long residence time) and soil fertility (soil conditioning effect). Intensive study of biochar-rich dark earths in the Amazon (terra preta), has led to a wider appreciation of biochar's unique properties as a soil enhancer.

To differentiate between the different pyrolysis reactors, nomenclature recommended by Emrich (1985) is given below.

- **Kiln:** Kilns are used in traditional biochar making, solely to produce biochar
- **Retorts and converters:** Industrial reactors that are capable of recovering and refining not only the biochar but also products from volatile fractions (liquid condensates and syngases) are referred to as retorts or converters
- **Retort:** The term retort refers to a reactor that has the ability to pyrolyzepile-wood, or wood log over 30 cm long and over 18 cm in diameter (Emrich, 1985).
- **Converters:** Produce biochar by carbonizing small particles of biomass such as chipped or pelletized wood.
- **Slow pyrolysis:** Refers to a process in which large biomass particles are heated slowly in the absence of oxygen to produce biochar.
- **Fast pyrolysis:** Refers to reactors designed to maximise the yields of bio-oil and typically use powdery biomass as feedstock.

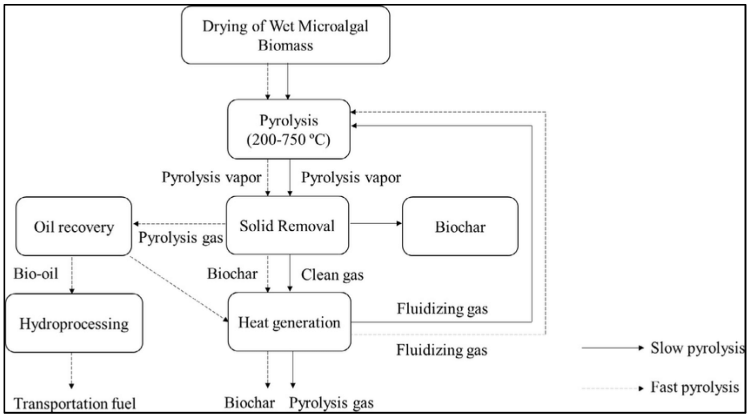


Fig 1: Biochar production

Biochar and Water Quality Remediation:

In aquaculture, pond soil and water quality have a direct effect on the health and productivity of cultured fish. The soil nutrients determine the plankton production and productivity of the pond. Alkaline soil or near-neutral soil promotes productivity. However, the fertilized acid soils are less productive compared to alkaline soil. Biochar has alkaline properties which can be used for improving the soil pH [3]. High organic matter and acidic soils reduce pond productivity. The carbon dioxide and ammonia gases are highly soluble in water and can, at high concentrations, toxic to the aquatic animals. The soil microorganisms convert the organic matter into carbon dioxide gases by oxidation. Among the different forms for nitrogen, unionized ammonia (NH_3) is a limiting factor and toxic to aquatic animals at a level above 0.02mg/l. The accumulation of feed and other organic material in the sediment favours anaerobic environment and release of ammonia. Biochar can remove the accumulation of toxic ammonia from the water [4]. Biochar prepared form water hyacinth was used for soil amendment which improved the growth rate of paddy in integrated fish culture system [5]. Biochar produced from rice husk and perlite as the growth substrate in hydroponics improved vegetable production and decreased algal growth [6]. Biochar treatment of transplanted lettuce (*Lactuca sativa*) and Chinese cabbage (*Brassica chinensis*) increased final biomass, root biomass, plant height [7]. In fisheries, ammonium affects the most as it acts as a water pollutant. It causes eutrophication of groundwater, aquaculture, and surface water. Rice straw is also an economic waste of rice, with millions of tons produced per year around the world. It has been observed that biochar produced from rice straw can be used effectively for ammonium removal from aqueous solutions. Biochar acts as a biofilter in fish culture. Addition of biochar to the fish production ponds decrease the level of heavy metals and reduced its accumulation in fish organs especially the sawdust biochar, followed by rice husk biochar.

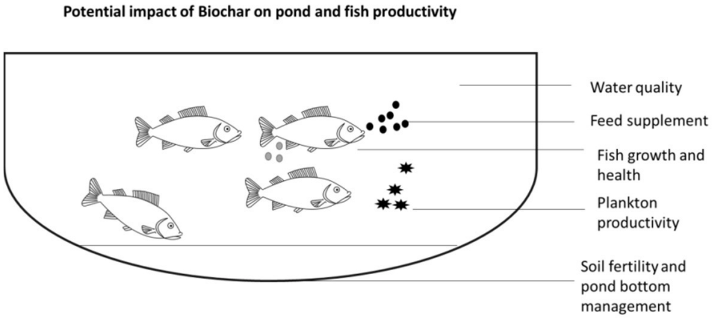


Fig 2: Potential applications of biochar in the aquaculture system

Biochar as Feed Supplement:

In poultry farming, biochar as a feed supplement at a 0.4 to 0.6% rate improves digestion and feed efficiency by increasing the energy absorption. The intestinal health and immune status also improved by feeding the biochar supplement. Biochar fed animals produce good fertilizer quality manure and reduce disease like footpad disease associated with litter management [8]. In aquaculture, this biochar manure can improve the pond bottom, soil quality, and prevent related disease outbreak. Biochar with molasses was fed directly to the cows to improve the soil fertility and farm productivity [9]. Biochar is applied as a feed supplement for goats, sheep, horses, and pigs. Biochar can improve the growth performance, productivity, and immune status of fish. Feeding biochar improves animal health, meat quality, and adsorbs toxins [10]. Biochar combined with silage, reduces mycotoxin formation, bind pesticides, suppress the butyric acid formation and enhance the number of lactic bacteria. Biochar with straw or sawdust bedding at 5–10% (vol) reduces hoof diseases, odours and nutrient losses. Biochar produced from water hyacinth at 0.5 to 1% supplement enhanced the growth rate of Tilapia (*Oreochromis mossambicus*) [5]. In brown trout (*Salmo trutta*) biochar application has improved the growth performance and survival rate at 2-3% supplementation [11]. Biochar at 0.5% supplement in feed improved growth rate and decreased the ammonia nitrogen excretion in flounder (*Paralichthys olivaceus*) [12]. Feeding biochar improved growth rate of *Pangasius hypophthalmus* [13]. To improve animal health, increase nutrient intake efficiency and thus to increase the productivity from the livestock, farmers use biochar as a feed supplement. The excreted nitrogen-rich biochar-manure becomes more valuable organic fertilizer causing lower nutrient losses and greenhouse gas emissions during storage and soil application. Wide range of toxic substances bound to the gastrointestinal tract due to the adsorption qualities of the biochar. It also leads to the detoxification of the lipophilic toxins in plasma through enteral dialysis. Dysbiosis and eubiosis can be effectively influenced by the use of biochar despite environmental fluctuations in the digestive tract. The adsorption qualities of biochar permit a wide range of toxic substances to be bound in the gastrointestinal tract. They also lead to the detoxification of already resorbed toxins (in particular lipophilic toxins) in the plasma via “enteral dialysis”. The oxidation and deamination of biogenic amines also play a particularly stabilising role in the intestines. Dysbiosis can be very efficiently and positively influenced by biochar, and eubiosis can be maintained much longer despite environmental fluctuations in the digestive tract.

Biochar - A Potential Chemical Adsorbent:

The chemical contaminants such as pesticides, herbicides from agricultural runoff, and heavy metals are toxic to aquatic animals. Biochar application has the potential to reduce the negative impacts of these chemicals in aquaculture [14]. Antibiotic use is most common in aquaculture farms, which accumulates in faeces, pond sediments, and other plants and transfer through the food chain. Biochar has the potential to adsorb the antibiotics from the water [15]. Different biochar compounds made from agricultural biowastes Eucalyptus bark, peanut shells, walnut shells, peach stones, grape seeds, and olive waste have reduced the concentration of fish anaesthetics MS-22 (Tricaine Methanesulfonate) [16]. The effluent treatment facility in aquaculture farms can utilize the adsorbent property of the biochar for water treatment which reduces the impact on the environment. Biochar also has a high affinity for organic pollutants and can be used as a low-cost adsorbent for the removal of environmental organic pollutants from the water environment. The contaminants include dyes, pesticides, herbicides, antibiotics, and other organic contaminants. Biochar helps in removal of ammonium, nitrate, and phosphate from aqueous solution to quantify its potential for nutrient pollution mitigation in aquatic ecosystems. The removal ability varies with different biochar feedstock sources as the mineral components originated from the feedstocks play an important role in the adsorption properties of biochar. Biochar has high adsorption efficiency for dye at alkaline pH due to the electrostatic interaction between the cationic molecule of dye and negatively charged biochar surface. However, biochar shows lower adsorption efficiency at acidic pH which may be due to the protonation and the presence of excess H+ ions that may compete with the cationic molecules for the adsorption sites available on biochar.

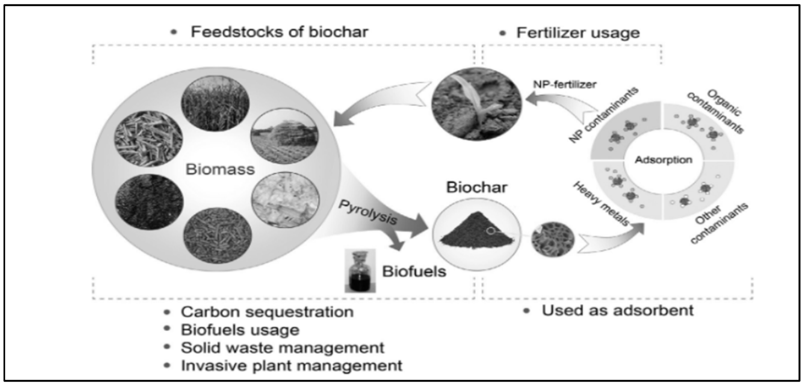


Fig 3: The benefits of biochar applied as an effective adsorbent for wastewater treatment.

Biochar and Fish Health

Aquaculture is one of the most important options in animal protein production and requires high quality feeds with high protein content as well as some complementary additives to keep organisms healthy and favour growth. It has been demonstrated that the use of biochar in fish feed significantly increases the bodyweight, specific growth rate (SGR), feed conversion ratio (FCR) and condition factor (CF). It was found that juvenile Japanese flounder (*Paralichthys olivaceus*) fed with 0.5% bamboo charcoal had significantly increased weight gain ($p < .05$), specific growth rate (SGR), FCR, and protein efficiency ratio (PER). Nitrogen content in feeds is an essential determinant factor to fish growth. The study conducted on GIFT tilapia to evaluate the performance of biochar feed. Fishes fed with 1% biochar incorporated diet showed the positive results by increasing the final weight, weight gain (WG), specific growth rate (SGR) and lowest feed conversion ratio (FCR). The productivity of the inland saline aquaculture can be increased by making use of biochar as it enhances the population of microflora and fauna. Biochar helps in reduction of leaching of nutrients from the system which leads to the reduction of stress in fish and improves the growth and health status of the fishes.

Properties of Biochar

The properties of biochar are influenced by the composition of the raw material such as the content of cellulose, hemicellulose and lignin, temperature, reaction time, and reactor type all contribute to the properties of the final products. Temperature is a crucial factor among all other affecting factors. The increased temperature also resulted in smaller biochar particles, and particle size is one of the major considerations in applying biochar as a feeding supplement in ruminant diets. The yield of biochar is dependent on the types of feedstock. Feedstock with high lignin content, which is related to the formation of char, would usually generate higher biochar yield. The activation of biochar is carried out to maximize its surface area and pore density and therefore increase its ability to capture pollutants and other particulates.

Post-Processing to Enhance Biochar Properties

The effectiveness of the biochar can be altered by post-processing the biochar.

Techniques can include:

- Treat biochar with phosphoric acid to enhance functional groups, reduce pH and make a slow-release phosphate fertiliser,
- Treating the biomass with alkali (e.g. Potassium hydroxide) to increase pH and increase K content
- Infuse the biochar with organic or inorganic nitrogen compounds e.g. Urine to enhance N content
- Mix with nutrient-rich organic material eg. manure. May need to heat, to sterilize and dry the mixture to eliminate biohazard and facilitate handling
- Add minerals e.g. Rock phosphate, gypsum, dolomite, iron oxides, lime to address specific soil constraints
- Add chemicals e.g. Urea, diammonium phosphate to make a compound fertilizer
- Granulate or pelletise to aid handling and biochar application

References

- Hagemann, N.; Spokas, K.; Schmidt, H.-P.; Kägi, R.; Böhler, M.; Bucheli, T. Activated Carbon, Biochar and Charcoal: Linkages and Synergies across Pyrogenic Carbon’s ABCs. *Water* 2018, *10*, 182.
- Elad, Y.; David, D.R.; Harel, Y.M.; Borenshtein, M.; Kalifa, H.B.; Silber, A.; Graber, E.R. Induction of Systemic Resistance in Plants by Biochar, a Soil-Applied Carbon Sequestering Agent. *Phytopathology* 2010, *100*, 913–921.

- Deem, L.M.; Crow, S.E. Biochar. In *Reference Module in Earth Systems and Environmental Sciences*; Elsevier, 2017; p. B978012409548910524X ISBN 978-0-12-409548-9.
- Khalil, A.; Sergeevich, N.; Borisova, V. Removal of ammonium from fish farms by biochar obtained from rice straw: Isotherm and kinetic studies for ammonium adsorption. *Adsorpt. Sci. Technol.* 2018, *36*, 1294–1309.
- Najmudeen, T.M.; Febna, M.A.A.; Rojith, G.; Zacharia, P.U. Characterisation of Biochar From Water Hyacinth Eichhornia crassipes and the Effects of Biochar on the Growth of Fish and Paddy in Integrated Culture Systems. *J. Coast. Res.* 2019, *86*, 225–234.
- Awad, Y.M.; Lee, S.-E.; Ahmed, M.B.M.; Vu, N.T.; Farooq, M.; Kim, I.S.; Kim, H.S.; Vithanage, M.; Usman, A.R.A.; Al-Wabel, M.; et al. Biochar, a potential hydroponic growth substrate, enhances the nutritional status and growth of leafy vegetables. *J. Clean. Prod.* 2017, *156*, 581–588.
- Carter, S.; Shackley, S.; Sohi, S.; Suy, T.; Haefele, S. The Impact of Biochar Application on Soil Properties and Plant Growth of Pot Grown Lettuce (Lactuca sativa) and Cabbage (Brassica chinensis). *Agronomy* 2013, *3*, 404–418.
- Gerlach, H.; Schmidt, H.-P. Biochar in poultry farming. 4.
- Joseph, S.; Pow, D.; Dawson, K.; Mitchell, D.R.G.; Rawal, A.; Hook, J.; Taherymoosavi, S.; Van Zwieten, L.; Rust, J.; Donne, S.; et al. Feeding Biochar to Cows: An Innovative Solution for Improving Soil Fertility and Farm Productivity. *Spec. Issue Appl. Biochars Soil Constraints Chall. Solut.* 2015, *25*, 666–679.
- Schmidt, H.-P.; Hagemann, N.; Draper, K.; Kammann, C. The use of biochar in animal feeding. *PeerJ* 2019, *7*, e7373.
- Khaki, N.D.; Malcevschi, A.; Voccia, A.; Marzano, F. Interaction Of Dietary Biochar (Black Carbon) On The Growth Performance And Survival Rate Of Early Stage Larvae Of Brown Salmo trutta. 2.
- Thu, M.; Koshio, S.; Ishikawa, M.; Yokoyama, S. Effects of Supplementation of Dietary Bamboo Charcoal on Growth Performance and Body Composition of Juvenile Japanese Flounder, Paralichthys olivaceus. *J. World Aquac. Soc.* 2010, *41*, 255–262.

- Lan, T.T.; Preston, T.R.; Leng, R.A. Feeding biochar or charcoal increased the growth rate of striped catfish (Pangasius hypophthalmus) and improved water quality. 5.
- M., S.; M, E.-B.; G., E.– S.; I., K. Evaluation of Biochar as Abio filter for Application in Commercial Fish Culture System. *Mediterr. Aquac. J.* 2014, *6*, 30–39.
- Wang, H.; Chu, Y.; Fang, C.; Huang, F.; Song, Y.; Xue, X. Sorption of tetracycline on biochar derived from rice straw under different temperatures. *PLOS ONE* 2017, *12*, e0182776.
- Ferreira, C.I.A.; Calisto, V.; Santos, S.M.; Cuerda-Correa, E.M.; Otero, M.; Nadais, H.; Esteves, V.I. Application of pyrolysed agricultural biowastes as adsorbents for fish anaesthetic (MS-222) removal from water. *J. Anal. Appl. Pyrolysis* 2015, *112*, 313–324.

13.Designer Biochar for Enhancing Aquaculture Productivity and Managing Associated Environmental Challenges

Manish Kumar, Aarti Ray, Adnan Asad Karim & N K Dhal
Environment & Sustainability Dept., CSIR-Institute of Minerals and Materials Technology,
Bhubaneswar-751013

Introduction

Traditionally, biochars are carbonaceous materials produced from diverse biomass through pyrolysis process, mainly targeted for its edaphic application. Nevertheless, in recent past, several researchers worldwide are exploring opportunity to utilise biochars for non-agricultural use like environmental application i.e. wastewater treatment, contaminated site remediation; feed materials for livestock; advanced materials for energy application; as construction materials etc.

Several literatures also highlights vast scope of biochar utilisation for aquaculture sector. Earlier research work majorly reported about effects of biochar on growth and development [body weight increase (BWI), specific growth rate (SGR), feed conversion ratio (FCR) and condition factor (CF)] of aquatic species along with changes in the quality of aqua sphere for benefit to commercial aquatic farming. Fishes were fed with biochar based supplements for improving fish health and productivity along with improving water quality. Ahmed Khalil (2018) reported about removal of toxic substances like ammonia etc. from fishery farms through adsorption by biochar. Despite these researches, there is still long way to go to fully utilise the potential of biochar in the aquaculture activities due to diversity of biomass which can be utilised for biochar production vis-à-vis production methods and processes. Now a days, designer biochar concept is growing which targets a specific function or role during aquafarming activities.

Designer Biochars and Role in Increasing Aquaculture Productivity

Biochars which are produced utilising selected biomass through identified methods and processes with specific characteristics for targeted purpose are regarded as "designer biochars." Engineered/designer biochars are commonly used terms to indicate application-oriented, outcome-based biochar modification or synthesis. Recent attention are also focused on modification of biochar with novel structures and surface properties in order to improve its utility for aquaculture. The basic schematic diagram given below reflects about the designer biochar concept. Although several designer biochars concept can be discussed, but the intention herewith is to highlight the work undertaken and planned at CSIR-Institute of Minerals & Materials Technology, Bhubaneswar.

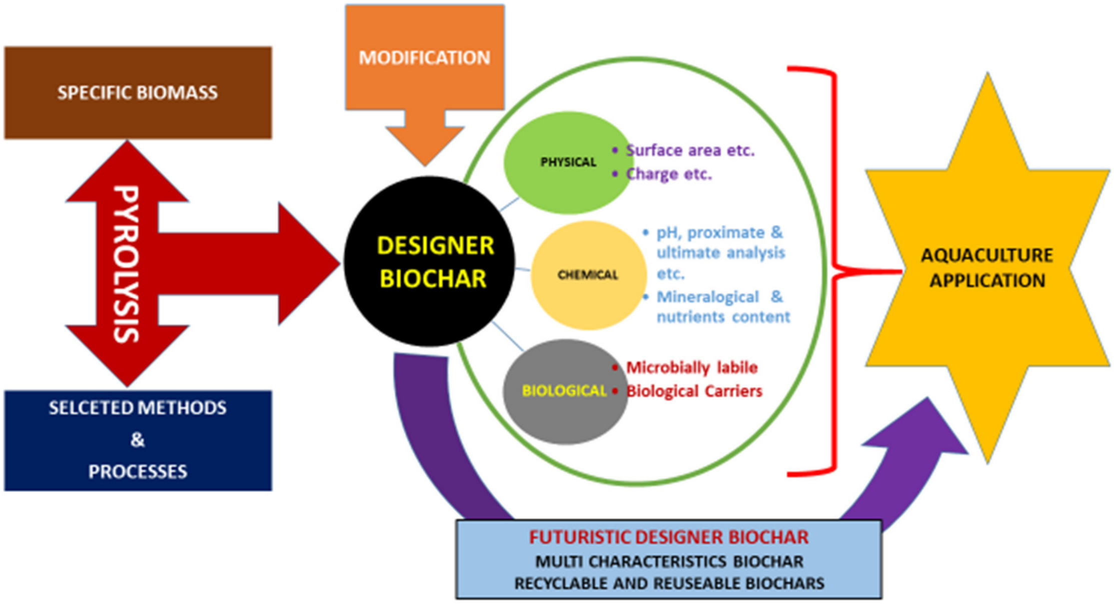


Fig 1. Schema of designer biochar towards aquaculture application

Nutrient Enriched Biochar for Aquaculture

Like any other living organism, one of the most important factor required for aquaculture flora & fauna are nutrients. In case of aquaculture, Nitrogen, phosphorus and potassium are the major nutrients and are mainly provided using chemical fertilisers. Among major nutrients, the importance of potassium in shrimp farming in salt water basins for building up the exoskeletons. Most commonly, the K requirement of aquaculture are fulfilled through Muriate of potash (potassium chloride, KCl) and sulphate of potash (potassium sulphate, K2SO4).

Potassium enriched biochar from banana peduncle waste was prepared through thermal plasma processing. The available potassium content in banana peduncle i.e. 66.6 g/kg was enriched up to 258 g/kg in plasma processed biochar. Potassium of banana peduncle was enriched due to its residualization and mineralization during the plasma processing. The K enriched biochar can be utilised as a potential source of available potassium with additional advantages of liming, soil conditioning and carbon sequestration in aquaculture.

Similarly, by selecting specific waste biomass and processing methods, nutrients enriched biochars can be developed useful specifically to aquaculture farming.

Designer Biochars and Role in Managing Aquaculture Associated Environmental Challenges:

Modern day aquaculture activities are intensive and faces severe challenges related to environment. Aquaculture activities generate wastewater with high in nutrients, where developing a proper treatment system is crucial. During the fish, crustaceans and even bivalves farming, waste in the form of fecal matter and unused feed are generated. These largely nitrogen-based wastes increase biological oxygen demand (BOD) and may lead to net loss of productivity. Also, use of antibiotics, anti-foulants, and pesticides are the major problems that aquaculture introduces to the environment.

Biochars and their modified forms as activated carbon are now a days utilised as intervention towards managing emerging environmental challenges related to aquaculture. The important characteristics of biochars which contribute for tackling pollution both in water and aquaculture sediment bed are physical properties like structure, surface area, porosity, charge dynamics etc., chemical features like pH, elemental and mineralogical composition etc. The figure given below highlights about the mechanism of pollution remediation by use of biochars for aquaculture.

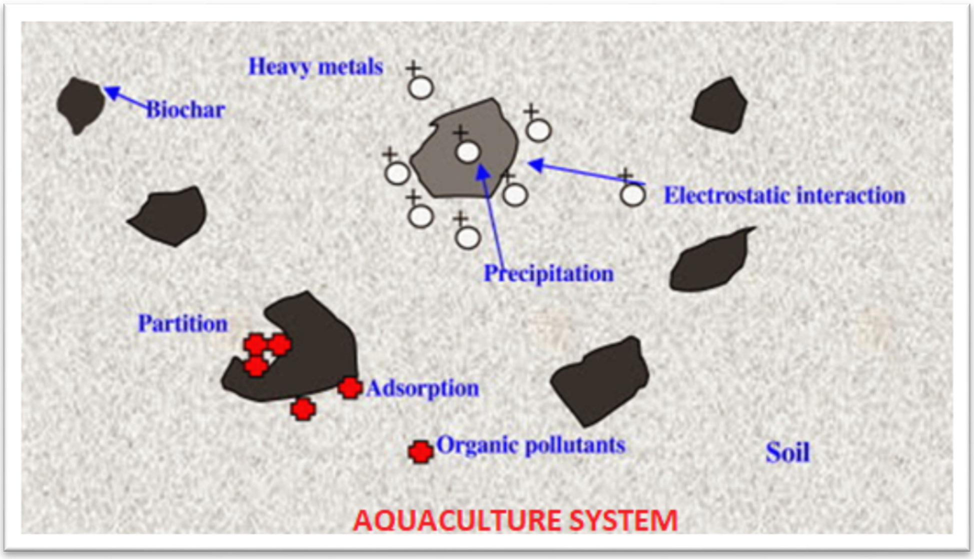


Fig. 2 Mechanism of pollution remediation through biochars from water and aqua-soil system

Biochar production methods regulates the properties of biochar produced from biomass. Slow pyrolysis and fast pyrolysis methods results into different type of biochar, which can be utilised for different application in aquaculture. pH of lower pyrolytic temperature produced biochar are relatively less alkaline and they can be utilised to supply of nutrients without affecting water and or sediment reaction to a great extent. In case of biochar utilised for pollution remediation based on their physico-chemical properties, the H: C ratio linked to aromaticity and O: C ratio linked to stability hold their importance. Biochars with lower H:C ratio and high fixed carbon are mainly recalcitrant (graphitic structure) and they can be utilised for aquaculture without having negative influence on water quality. The basic differences between biochar and designer biochar are tabulated below

Table 1: Biochar Vs Designer Biochar for aquaculture application

PARAMETERS	BIOCHAR	DESIGNER BIOCHAR
Production Process	Pyrolysis	Pyrolysis, co-pyrolysis
Material Classification	Carbonaceous	Carbonaceous and their composites
Crystallinity	Amorphous	Amorphous/Graphitic/ Crystalline
Porosity	Porous (mainly macro)	Porous (mainly meso/micro)
Feedstock	Any organic waste preferably lignocellulosic waste	Any organic waste and or inorganic materials etc.
Modification	Physical or chemical modification is not compulsory	Physical/chemical/biological modification are required
Recalcitrant	Low to moderate	High
Surface Area	Low	High
Nutrients Content	Generally low	Relatively high
Application	Mainly used for soil amendment	For aquatic feed, environmental remediation and management etc.

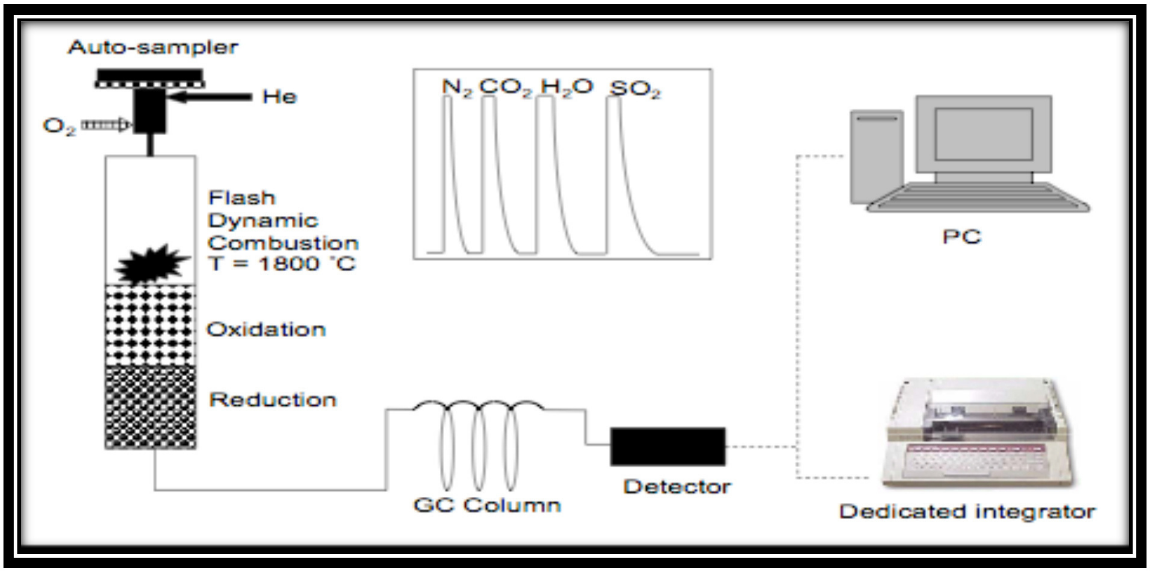
Although some advancement in biochar research is supporting its utilisation for aquaculture sector, but still more works are needed towards its wider applicability. Low cost and high efficient designer biochar will contribute for sustainable development of aquaculture sector in coming years.

14. Analysis of Biochar Sample by CHNS

Vidya Shree Bharti, Ashutosh Kumar Singh & Vijay Kumar A
ICAR- Central Institute of Fisheries Education, Versova, Mumbai-400061

Introduction

The analyzer operates according to the dynamics of the flash combustion of the sample. The sample is weighed in a tin or silver capsule and introduced into the combustion reactor where with the proper amount of oxygen and combustion catalysts, the sample is combusted. After combustion, the reaction gas products (CO_2, H_2O, NO_x, and SO_2) are carried by helium flow to a copper reactor where excess O_2 is consumed (CuO) and NO_x products are converted to N_2. The products are carried through a packed GC column that provides the separation of the combustion gases and is detected by a TCD detector.



The combustion products are swept out of the combustion chamber by an inert carrier gas such as helium and passed overheated (about 600^o C) high purity copper. This copper can be situated at the base of the combustion chamber or in a separate furnace. The function of this copper is to remove any oxygen not consumed in the initial combustion and to convert any oxides of nitrogen to nitrogen gas. The gases are then passed through the absorbent traps to leave only carbon dioxide, water, nitrogen, and sulfur dioxide.

Detection of the gases can be carried out by a GC separation followed by quantification using thermal conductivity detection.

CHNS Instrumentation

All instruments require two gas supplies: (i) an inert carrier gas (helium recommended); and (ii) high purity oxygen (minimum 99.9995%). Additionally, GC-type gas filters are also usually fitted to prevent trace organic species and water entering the combustion system. For solids or viscous liquids, samples are weighed out into tin capsules; for liquids, samples can be sealed in individual aluminum vials or introduced via a liquid auto-sampler. The combustion section of the analyzer is designed to achieve both complete combustion of the sample and conversion of oxides of nitrogen to nitrogen gas (N_2).

The combustion section of the analyzer is designed to achieve both complete combustion of the sample and conversion of oxides of nitrogen to nitrogen gas (N_2). Both the catalysts/absorbents and copper metal are packed into readily exchangeable tubes made of ceramic material or high-quality silica. the oxygen is added to the tube at the same time as the sample introduction and continues to flow for a set time. With small test portions, the combustion gases can be separated on a GC column and quantified using a thermal conductivity detector, another important consideration is the amount of ash that is formed during the combustion and its removal. The ash will comprise the remains of tin and aluminium containers and the inorganic residues from the test portion.

Operation of CHNS Analyser

- Turn on the gas cylinder,carrier gas-helium(1.2bar) and combustion gas O_2(2.5 bar)
- Switch on the green bottom of the instrument at right hand side.
- Switch on the computer,open the software: Vario MICRO
- Adjust the pressure, by turning the control knob on gas cylinders.
- On the monitor check for-
    - Pressure:1200(+/-50mbar)
    - MFC TCD:200(+/-5)mi/min
    - Flow Helium: 200(+/-10)ml/min
    - Flow O_2: 10(+/-2)ml/min when no combustion and 30(+/-2)ml/min during onset of combustion

- Temperature setting :GO to options →setting→parameters
    - Combustion tube→1150°c
    - Reduction tube→550°c
- Let TCD temperature come to 59-60°c automatically
- Give reference run-confirm all carousels are free of samples
- Run atleast 3-5 dummy,3-5 blank at every use.
- Then run 2-3 Run in,3-5 standards (sulfanilic acid/sulphanilamide) (enter correction factor after standards:go to Math→Factor→Yes)
- Samples are weighed in micro-weighing balance and packed in tin boats and introduced into carousels
- After introducing all the samples,give the appropriate method and start Auto-run
- After use at the end of the day, run 3-5 more blanks.
- Reduce temperature to < than 300°c,before turning off the instrument
- When instrument temperature reaches below 300°c, save data and turn off the computer
- Switch off the green side button on the instrument.
- Turn off the Helium gas.
- Cover the instruments and computer.

15. Basic Statistical Technique

Vinod Kumar Yadav & Vidya Shree Bharti
ICAR- Central Institute of Fisheries Education, Versova, Mumbai-400061

Descriptive Statistics

Descriptive statistics is the term given to the analysis of data that helps describe, show or summarize data in a meaningful way such that, for example, patterns might emerge from the data. Descriptive statistics do not, however, allow us to make conclusions beyond the data we have analysed or reach conclusions regarding any hypotheses we might have made. They are simply a way to describe our data.

Descriptive statistics are very important because if we simply presented our raw data it would be hard to visualize what the data was showing, especially if there was a lot of it. Descriptive statistics therefore enables us to present the data in a more meaningful way, which allows simpler interpretation of the data. For example, if we had the results of 100 pieces of students' coursework, we may be interested in the overall performance of those students. We would also be interested in the distribution or spread of the marks. Descriptive statistics allow us to do this. Typically, there are two general types of statistic that are used to describe data:

Measures of Central Tendency

These are ways of describing the central position of a frequency distribution for a group of data. In this case, the frequency distribution is simply the distribution and pattern of marks scored by the 100 students from the lowest to the highest. We can describe this central position using a number of statistics, including the mode, median, and mean (Arithmetic Mean, Geometrical Mean & Harmonic Mean).

Measures of Relative Dispersion

Suppose that the two distributions to be compared are expressed in the same units and their means are equal or nearly equal. Then their variability can be compared directly by using their standard deviations. However, if their means are widely different or if they are expressed in different units of measurement, we can not use the standard deviations as such for comparing their variability. We have to use the relative measures of dispersion in such situations. There are relative dispersions in relation to range, the quartile deviation, the mean deviation, and the standard deviation. Of these, the coefficient of variation which is related to the

$$\text{C.V.} = (\text{S.D.} / \text{Mean}) \times 100.$$

The C.V. is a unit-free measure. It is always expressed as percentage. The C.V. will be small. if the variation is small of the two groups, the one with less C.V. is said to be more consistent.

Skewness and Kurtosis

The average and measure of dispersion can describe the distribution but they are not sufficient to describe the nature of the distribution. For this purpose we use other concepts known as Skewness and Kurtosis.

Skewness is a measure of symmetry, or more precisely, the lack of symmetry. A distribution, or data set, is symmetric if it looks the same to the left and right of the center point.

Kurtosis is a measure of whether the data are peaked or flat relative to a normal distribution. That is, data sets with high kurtosis tend to have a distinct peak near the mean, decline rather rapidly, and have heavy tails. Data sets with low kurtosis tend to have a flat top near the mean rather than a sharp peak.

When we use descriptive statistics it is useful to summarize our group of data using a combination of tabulated description (i.e., tables), graphical description (i.e., graphs and charts) and statistical commentary (i.e., a discussion of the results).

Inferential Statistics

As the normal distribution is very important for statistical inference point of view so it is desired to examine the assumption to test whether the data is from a normal distribution. The normality can be tested by plotting a normal plot. In a normal probability plot each observed value is paired with its expected value from the normal distribution. In a situation of normality, it is expected that points will fall on straight line. In addition to this a plot of deviation from straight line can also be plotted as detrended normal plot. A structure-less detrended normal plot confirms normality. These two plots give a visual basis for examining normality. Besides these visual displays, the statistical tests are Shappiro-Wilks and the Lilliefors. The Lilliefors test is based on the modification of the Kolmogorov-Smirov test for the situation when means and variances are not known but are estimated from the data. The Shapiro-Wilks test is more powerful in many situations as compared to other tests.

We have seen that descriptive statistics provide information about our immediate group of data. For example, we could calculate the mean and standard deviation of the exam marks for the 100 students and this could provide valuable information about this group of 100 students. Any group of data like this, which includes all the data you are interested in, is called a population. A population can be small or large, as long as it includes all the data you are interested in. For example, if you were only interested in the exam marks of 100 students, the 100 students would represent your population. Descriptive statistics are applied to populations, and the properties of populations, like the mean or standard deviation, are called parameters as they represent the whole population (i.e., everybody you are interested in).

Hypothesis Testing

Significance testing is used to help make a judgment about a claim by addressing the question, Can the observed difference be attributed to chance? We break up significance testing into three (or four) steps:

Step A: Null and alternative hypotheses

The first step of hypothesis testing is to convert the research question into null and alterative hypotheses. We start with the **null hypothesis (H_0)**. The null hypothesis is a claim of "no difference." The opposing hypothesis is the **alternative hypothesis (H_1)**. The alternative hypothesis is a claim of "a difference in the population," and is the hypothesis the researcher often hopes to bolster. It is important to keep in mind that the null and alternative hypotheses reference population values, and not observed statistics.

Test of Significance for Small Samples

Tests Based on t-distribution

Assumptions w.r.t. t tests

- The sample must be chosen randomly
- The data must be quantitative (measurable e.g height, BP)
- The data should follow normal distribution
- The size is ideally <30 in each group
- Populations should have equal variance
- The t-test used must be appropriate for the design. Paired t for the paired design and Unpaired t for comparing two independent group means.

Paired t-test

- Paired t-test is used whenever the two populations are related
- Generally used when measurements are taken from same subjects/units before and after administering some treatment/drug etc.
- Test BP before and after administering some experimental drug
- Sometime, the subjects may be different but are related through some other criteria
- Eg. Husband and Wife

Chi-square Distribution

- Distribution of sum of square of n independent standard normal variate is known as χ^2 -distribution with n d.f
- The Chi –square distribution has only one parameter called degree of freedom
- The Shape of chi-squared distribution curve is skewed to the right for small d.f.
- The entire chi-square distribution curve lies to the right of vertical axis
- It assumes non –negative value only

Application

- To test if the hypothetical value of the population variance is $\sigma^2 = \sigma^2_{0(\text{say})}$
- To test the goodness of fit

- To test the independence of attribute
- To test the homogeneity of independence estimate of population variance

A Goodness-of-Fit Test

- Used for testing the discrepancy between the expected and observed frequency
- It enables us to find if the deviation of experiment from the theory is just by chance or is it really due to inadequacy of the theory to the observed data
- H_o = The fitted distribution is good fit the given data
- H_1= not a good fit

The frequencies obtained from the performance of an experiment are called the observed frequencies and are denoted by O and expected frequencies denoted by E is the frequencies that we expect to obtain if the null hypothesis is true. The expected frequency for a category is obtained by E=np where n is the sample size and p is the probability that element belong to that category if the null hypothesis is true

- The test statistic for a goodness-of-fit test is $\chi^2 = \sum(O-E)^2 /E$
- Where O=observed frequency for a category
- E= Expected frequency for a category=np
- Remember that a chi-square goodness-of-fit test is always right-tailed

2.1.8 F-distribution

Definition

If X^2_1 and X^2_2 are two independent chi-square variate with v_1 and v_2 df then F-statistic is defined by

$F = (X^2_1/v_1)/(X^2_2/ v_2)$

Steps Required to Compute an *f* Statistic:

- Select a random sample of size n_1 from a normal population, having a standard deviation equal to σ_1.
- Select an independent random sample of size n_2 from a normal population, having a standard deviation equal to σ_2.

- The *f* statistic is the ratio of s_1^2/σ_1^2 and s_2^2/σ_2^2
- The following equivalent equations are commonly used to compute an *f* statistic:
- $f=[s_1^2/\sigma_1^2]/[s_2^2/\sigma_2^2]$
- $f = [X_1^2 / v_1] / [X_2^2 / v_2]$

- where σ_1 is the standard deviation of population1, s_1 is the standard deviation of the sample drawn from population1, σ_2 is the standard deviation of population2, s_2 is the standard deviation of the sample drawn from population 2, X^2_1 is the chi-square statistic for the sample drawn from population 1, v_1 is the degrees of freedom for X_1^2, X_2^2 is the chi-square statistic for the sample drawn from population 2, and v_2 is the degrees of freedom for X_2^2 . Note that degrees of freedom $v_1 = n_1 - 1$, and degrees of freedom $v_2 = n_2 - 1$.
- The distribution of all possible values of the *f* statistic is called an F distribution, with $v_1 = n_1 - 1$ and $v_2 = n_2 - 1$ degrees of freedom.
- The curve of the F distribution depends on the degrees of freedom, v_1 and v_2. When describing an F distribution, the number of degrees of freedom associated with the standard deviation in the numerator of the *f* statistic is always stated first.
- Thus, f (5, 9) would refer to an F distribution with $v_1 = 5$ and $v_2 = 9$ degrees of freedom; whereas $f(9, 5)$ would refer to an F distribution with $v_1 = 9$ and $v_2 = 5$ degrees of freedom. Note that the curve represented by $f(5, 9)$ would differ from the curve represented by $f(9, 5)$.

Application of F-distribution

- F-test for equality of two population variances
- F-test for testing equality of several means (ANOVA technique)
- F-test for testing the significance of an observed multiple correlation coefficient
- F-test for testing the significance of an observed sample correlation ratio
- Test for equality of two population variances

Correlation

Correlation Measures the strength (degree) of linear relationship between paired two numerical variables. If the change in one variable affects a change in other variable, variable are said to be correlated.Pearson's Sample Correlation Coefficient, measures the direction(+ or -) and the strength of the linear association between two numerical paired variables.

Positive correlation

- Two variable deviate in the same direction. Typically, in the summer as the temperature increases people are thirstier.
- Height & wt of a group of persons, income & expenditure

Negative correlation

- Two variable deviate constantly in the opposite direction
- Price & demand of a commodity, Volume & pressure of a perfect gas.

Regression

If two variables are significantly correlated, it is possible to predict values of one variable from the other. This observation leads to a very important concept known as 'Regression Analysis'.Regression analysis, in general sense, means the estimation or prediction of the unknown value of one variable from the known value of the other variable. Regression analysis is a mathematical measure of the average relationship between two or more variables in terms of the original units of the data."

Study the nature of relationship by formulating statistical models which enables prediction of one variable called the dependent variable from the known value of others

References

- Chatfield, C. and Collins, A.J. (1990). Introduction to multivariate analysis. Chapman and Hall publications.
- Hair, J. F., Black, W. C., Babin, B. J., Anderson, R. E., & Tatham, R. L. (1995). 4th edition. New York: Macmillion Publishing Company. Chapter 3 Multiple Regression

- Johnson, R.A. and Wichern, D.W. (1996). Applied multivariate statistical analysis. Prentice-Hall of India Private Limited.

Participant List for Training (Biochar for Enhancing the Aquaculture Productivity).

Sl. No.	Name of the participants	Gender	Address
1.	Pradeep J.Yadav	Male	Pune
2.	Shushma Kumari	Female	Mazzafferpur, Bihar
3.	Miss Gagandeep Kour	Female	Kathua, jammu and Kashmir
4.	Monalisa Sarkar	Female	Muzaffarpur, Bihar
5.	Anam Shaikh	Female	Mumbai
6.	Alisha Rai	Female	Shahdol, Madhya Pradesh
7.	Mr. Shailendra Indulkar	Male	Vile Parle Mumbai ,MS
8.	Conrad M. Nunes	Male	Mahim, Mumbai
9.	Ananta Singh Ray	Male	Navi Mumbai
10.	Avinash Kumar	M	Bihar
11.	Atul Raosaheb Sathe	Male	Solapur, Maharashtra
12.	Devarsh Sudhakar Bhoir	M	Palghar,Maharastra
13.	Sonu Kumar	Male	Samastipur Bihar

Skill Development Programme
on
"Biochar for Enhancing the Aquaculture Productivity"

Date	Time	Lecture /practical	Faculty	Venue
3.3.2020	10.00-10.30 hrs	Registration of participants and pre-evaluation test	GT, VSB, SK	AEM Classroom
	10.30-11.00 hrs	Visits to different laboratories	VSB, SK,	CIFE Facility
	11.00-11:30 hrs	Overview of health management in aquaculture	KVR	AEM Classroom
	11.30-13.00 hrs	Importance of biochar in aquatic environment	VSB	AEM Classroom
	14.00-15.00 hrs	Physical and chemical characterization of biochar	VSB	AEM Classroom
	15.00-17.00 hrs	Preparation of biochar and pH & EC estimation	VSB, VK	Wetlab of old campus CIFE
4.3.2020	10.00-11.00 hrs	Impact of biochar on primary productivity	VSB	AEM Classroom
	11.00-13.00 hrs	Estimation of C:N ratio of biochar	VSB, VK	AEM Instrumentation Lab
	14.00-17.00 hrs	Visit to CIRCOT	VSB	CIRCOT, Matunga
5.3.2020	10.00-11.30 hrs	Bioremediation in aquaculture system through biochar	RB	AEM classroom
	11.30-13.00 hrs	Biochar for pollutant removal	SK	AEM Classroom
	14.00-15.00 hrs	Carbon sequestration in aquatic environment	SPS	AEM Classroom
	15.00-17.00 hrs	Statistical tools for data analysis	VKY	AEM Classroom
6.3.2020	10.00-11.30 hrs	Impact of biochar on sediment quality	VSB, AK	AEM Classroom
	11.30-13.00 hrs	Removal of ammonia through biochar	VSB, IJ	AEM Chemistry lab
	14.00-15.00 hrs	Biochar nutrient complex	VSB, IJ	AEM Classroom
	15.00-17.00 hrs	Analysis of Potassium in biochar nutrient complex	VSB, IJ	AEM Chemistry lab
7.3.2020	1.00-11.00 hrs	Biochar and its effects in mitigating greenhouse gas emission	VSB, VK	AEM Classroom
	11.00-13.00 hrs	Designer biochars for enhancing aquaculture productivity and managing associated environmental challenges	MK	AEM Classroom
	14.00-15.00 hrs	Impact of biochar on fish health	GT	AEM Classroom
	15.00-16.00 hrs	Carbon and its fraction	VSB, VK	AEM Classroom
	16.00-16.30 hrs	Post training evaluation	GT, SK,	AEM Classroom
	16.30-17.00 hrs	Valedictory Programme		

Skill Development Programme
on
"Biochar for Enhancing the Aquaculture Productivity"

Tea Break: 11.15-11.30 AM
15.15-15.30 PM

Lunch Break: 13.00-14.00 PM

KVR	Dr. K.V. Rajendran
GT	Dr. Gayatri Tripathi
SPS	Dr. Satya Prakash Shukla
VSB	Dr. Vidya Shree Bharti
MK	Dr. Manish Kumar
VKY	Dr. Vinod Kumar Yadav
SK	Dr. Saurav Kumar
RB	Dr. Rathi Bhuvneswari
IJ	Mrs. Iffat Jahan
AK	Mr. Ashutosh Kumar
VK	Mr. Vijay Kumar

Resource Persons

Sl. No	Name of Faculty	Designation	Email-ID
1.	Dr. K. V. Rajendran	Principal Scientist & Head AEHMD	kvrajendran@cife.edu.in
2.	Dr. Gayatri Tripathi	Principal Scientist	gayatrit@cife.edu.in
3.	Dr. S.P. Shukla	Principal Scientist	spshukla@cife.edu.in
4.	Dr. V.S. Bharti	Senior Scientist	vsbharti@cife.edu.in
5.	Dr. V. K. Yadav	Scientist	vinodkumar@cife.edu.in
6.	Dr. Manish Kumar	Scientist E-2	manish@immt.res.in
7.	Dr. S. Kumar	Scientist	saurav@cife.edu.in
8.	Dr. R. Bhuvneswari	Scientist	rathi@cife.edu.in

ICAR-CIFE

ICAR-Central Institute of Fisheries Education (CIFE), in over 50 years of existence, has emerged as a centre of excellence in higher education in fisheries and allied disciplines. The Institute was established on 6 June 1961, under the Ministry of Agriculture, Govt. of India with assistance from FAO/UNDP. It came under the administrative control of Indian Council of Agricultural Research (ICAR) in 1979. Considering the wide mandate involving education, research and extension and recognizing the pivotal role played by CIFE in human resources development in fisheries, the institute was conferred the status of Deemed-to-be-University in 1989. The institute with its core strength in quality teaching, research and training has become a brand name in fisheries higher education.

AEHM Division

Aquatic Enviornemnt and Animal Health Management Division conducts academic programmes and carry out research under two broad areas namely Aquatic Enviornmental Management (AEM) and Aquatic Animal Health Management (AAHM). Accordingly, the division offers M.F.Sc. and Ph.D. programmes in two disciplines, i.e. AEM and AAHM. The division also conducts demand-driven training programmes for farmers, entrepreneurs and researchers in the specialized areas, besides providing technical support and consultancy services to various stakeholders.

Training Schedule

Skill Development Programme
on
"Biochar for Enhancing the Aquaculture Productivity"

Programme Director

Dr. Gopal Krishna
Director/Vice-Chancellor
ICAR-CIFE, Mumbai

Course Director

Dr. K. V. Rajendran
Principal Scientist & Head
AEHM Division
ICAR-CIFE, Mumbai

Course Coordinators

Dr. Vidya Shree Bharti
Senior Scientist, AEHM Division
ICAR-CIFE, Mumbai

Dr. Gayatri Tripathi
Principal Scientist, AEHM Division
ICAR-CIFE, Mumbai

Dr. Saurav Kumar
Scientist, AEHM Division
ICAR-CIFE, Mumbai

Organized by

Aquatic Environment & Health Management Division
ICAR-Central Institute of Fisheries Education
(Deemed University), Mumbai

Under the aegis of
National Agricultural Higher Education Project (NAHEP)