

SILICA GEL EXTRACTION FROM RICE HUSK, ITS APPLICATIONS IN ION EXCHANGE CHROMATOGRAPHY, ANTI-MICROBIAL ACTIVITY AND ANTI-FUNGAL ACTIVITY.

Harshada Girish Toraskar, Kajal Pandit Lamane, Shruti Suryakant Bhujbal

Ty Biotechnology

Tilak College of Science and Commerce, Vashi

ABSTRACT

The husk of rice, in large quantities, is an easily available agricultural residue in India. It is a raw material which is rich in silica comprising about 90-98% silica. Due to its large ash content and the presence of sodium silicate in its ash, rice husk becomes an economical source to extract silica from the rice husk ash, which has an extensive market and also helps in ash removal. The husk of rice is a widely popular boiler fuel and the ash produced has to be disposed off properly. The process of extraction of silica not only offers a solution for waste clearance, but also recovers a valuable product. The surface area

of Silica gel has high specificity which allows it to adsorb water readily, making it suitable drying agent in various purposes. Silica is also used in ion exchange chromatography. Moreover, silica's inherent anti-fungal and anti-bacterial properties makes it an anti-fungal and anti-microbial agent. In this study, an attempt is made to extract and characterize amorphous silica, from rice husk ash, for its potential use in the food industry. At the same time, resolving the disposal issue of rice husk ash and safeguarding the environment from pollution.

INTRODUCTION

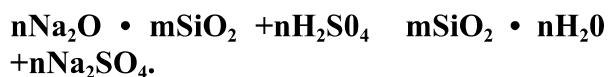
In today's times, when we are facing an ever-increasing crunch in every aspect of our lives and running out of resources to find sustainable solutions, the answer lies in waste, as our primary resource. We have waste in abundance in every nook and corner, but without its judicious utilization, there cannot be sustainable development. Food waste along with its by-products that aren't edible, contain many valuable products which may not only give us green alternatives, but also benefit the economy, especially of the developing nations.

Rice is the most prominent crop of India as it is the staple food for most of the people of the country. This crop is the backbone of livelihood for millions of rural households and plays a vital role in the country's food security, so the term "rice is life" is most appropriate in the Indian context. India occupies an important position both in area and production of rice. By the adoption of improved production technologies such as high-yielding varieties/ hybrids, expansion of irrigation potential, and use of chemical

fertilizer, supply of rice in the country has kept pace with the increase in demand. Demand for rice is expected to further increase in future as population is continuously increasing, so production of rice also needs to be increased. There is a need to further increase rice productivity because land area under rice cultivation is declining. Major constraints for productivity and sustainability of rice-based systems in the country are the inefficient use of inputs (fertilizer, water, labour), increasing scarcity of water and labour especially for rice cultivation, new emerging challenges from climate change, rising fuel prices, increasing cost of cultivation, and socioeconomic changes such as migration of labour, urbanization, less liking for agricultural work by youths, and concerns from environmental pollution. The only way to sustain rice production for meeting the increasing population demand is to increase the productivity per unit of area of rice with enhanced resource use efficiency. Rice milling generates a by-product known as husk. This surrounds the paddy grain. During milling of paddy about 78 % of

weight is received as rice, broken rice and bran. The Remaining 22 % of the weight of paddy is received as husk. This husk contains about 75 % organic volatile matter and the balance 25 % of the weight of this husk is converted into ash during the firing process, known as rice husk ash (RHA). This RHA in turn contains around 85 % - 90 % amorphous silica.

Silica gel, chemical formula: $m\text{SiO}_2 \cdot n\text{H}_2\text{O}$, appears as a slightly transparent White solid substance. It belongs to the genus amorphous. Silica is a highly active, porous material with large internal area. The specific surface area per silica can reach above 450 m. Therefore, it has strong absorption capability on the liquid or gas. Silica gel can't dissolve in water, not soluble in other solvents except hydrofluoric acid and strong alkali. The silica gel can be manufactured through the reaction between water glass and sulphuric acid, and further gelling, washing, desalting, animation, drying and screening. The reaction is as follows:



It has been found that industrial silica gel has 85 to 95 % mechanical strength, water content being below 2% and the moisture absorption rate of 25 to 30%.

- Gel silica is mainly used for gas absorption or drying, liquid dehydration and liquid chromatography, also used as catalysts or catalyst carriers.
- The textile industry uses it as a sizing agent. The drying and purification of gas is done by pore silica, the dehydration and purification of organic products, also used as a supported carrier.
- Discolouration gel is mainly used to indicate the relative humidity of airtight packaging, precision instruments and the inner space of instrument as well as being used for the moisture proof maintenance of precision instrumentation.

Ion exchange chromatography separates on the basis of charge. It is frequently chosen for the separation and purification of proteins, peptides, nucleic acids, polynucleotides and other charged molecules, mainly because of its high resolving power and high capacity.

However, Ion exchange chromatography must be done in conditions that are one unit away from the isoelectric point of a protein.

Anti-microbial activity is a term for all active principles(agents) that inhibit the growth of bacteria, the formation of microbial colonies, and may destroy microorganisms. The two methods used are **Agar cup method** and **Disc diffusion method**.

1. In **agar cup diffusion** assay different antibiotics to be assayed are added to the cup in the agar seeded with bacterial strains. After incubation, the zone of inhibition is observed.
2. The basic principle of the **disc diffusion method** is that as the antibiotic diffuses into the medium, a declining concentration gradient is formed, as the distance from the disc increases. The organism grows till it reaches an area where the antibiotic is no longer effective. At this point, a zone edge is formed.

Anti-fungal activity is a collective term for all active principles(agents) that inhibit fungal growth, the formation of microbial colonies, and may destroy microorganisms.

LITERATURE REVIEW

Rice husk is an agricultural byproduct, i.e., an unavoidable food waste, which is abundantly available in rice producing countries. India produces nearly 12 million tons of rice husk annually. Rice husk has a high ash varying from 18-20%. A major problem for rice growers is the disposal of rice husks. Until now they are discarded either by open incineration or burying. Uncontrolled burning of rice husk causes the ash, which is principally silica, to be converted into crystalline form and also renders it less reactive. Burning rice husk as fuel to generate energy results in the waste product, rice husk ash (RHA). RHA is rich in silica (**about 90-98%**) and can be an economically viable raw material for production of silica gels and powders (**Kamath and Proctor, 1998; Chakraverty and Kale- emullah, 1991**). RHA has been evaluated as an adsorbent of minor vegetable oil components (**Proctor et al., 1995; Proctor and Palaniappan, 1990**). Although various uses for rice hull and RHA

have been suggested in the literature, their disposal or utilization remains a major concern. RHA usually contains more than 60% silica (SiO_2), 10-40% carbon with minor mineral composition. Rice husk ash has a relatively high content of inorganic compounds, representing approximately 20% of the dry weight of the husk. Silica represents 94% of the total while the remaining 6% are K₂O, CaO, MgO, Al₂O₃, and P₂O₅ in decreasing concentrations. Silica (SiO_2) is a basic raw material that is widely used in electronics, ceramic, and polymer material industries. Because of its particle diameter, ultra fine silica powders have many technological applications, such as thixotropic agents, thermal insulators, composite fillers, etc. Silica also has been used as a major precursor for a variety of inorganic and organometallic materials which have applications in synthetic chemistry as catalysts, and in thin films or coatings for electronic and optical materials. (A.Umesh et al /Int.J. ChemTech Res. 2014)

MATERIALS FOR EXTRACTION OF SILICA GEL

SE RI AL NO .	PARTICULA RS	VOLUM E	QUANTI TY
1	Sample: Rice Husk Ash		
2	Reagents: 1 N HCl 1 N NaOH 1N H_2SO_4 Ethanol Distilled Water	500 ml 500 ml 20 ml 50 ml 500 ml	
3	Glasswares: Conical flask Beakers Pipettes Centrifuge Tubes	150 ml 200 ml 10 ml	5 5 5 10

SE RI AL NO .	PARTICULA RS	VOLUM E	QUANTI TY
4	Miscellaneous: Mortar Pestle Centrifuge Oven Induction		1 1 1 1
5	Others: Filter Paper pH paper Foil paper		1 1 1

MATERIALS FOR ION EXCHANGE CHROMATOGRAPHY

SE RI AL N O.	PARTICULAR S	VOLUM E	QUANTI TY
1	Sample: Extracted Silica Gel	5 g	
2	Reagents: HNO ₃ Aqueous solution of malachite green 2M HCL	1 ml 20 ml 20 ml	
3	Glassware: Burette Beaker		1 1
4	Miscellaneous: Burette Holder Burette clamp		1 1

MATERIALS FOR ANTI-MICROBIAL For Agar Cup

SE RI AL NO .	PARTICULAR S	VOLUM E	QUANTI TY
1	Sample: Extracted Silica Gel	3g	1
2	Reagents: Saline water	2ml	1
3	Culture: <i>S.aureus</i> <i>E.coli</i> <i>K.pneumoniae</i>	0.1ml 0.1ml 0.1ml	1 1 1
4	Glassware: St. MH agar butt St. Petri Plate St. Pipette St. Test tubes	25 ml	1 8 8 8
5	Miscellaneous: Alcohol Disinfectant dish Cork borer	5ml	1 1 1

For Disk Diffusion

SE RI AL NO .	PARTICULAR S	VOLUM E	QUANTI TY
1	Sample: Extracted Silica Gel	VOLUM E	QUANTI TY
2	Reagents: Saline water	3g	1
3	Culture: <i>S.aureus</i> <i>E.coli</i> <i>K.pneumoniae</i>	0.1ml 0.1ml 0.1ml	1 1 1
4	Paper disc: Gentamicin (10 mg)		1

SE RI AL NO .	PARTICULAR S	VOLUM E	QUANTI TY
5	Glassware: St. MH agar butt St. Petri Plate St. Pipette St. Test tubes	25 ml	1 8 8 8
	Miscellaneous: Disinfectant dish Cork borer Cotton Swab	5ml	1 1 1

MATERIALS FOR ANTI-FUNGAL

SE RI AL NO .	PARTICULAR S	VOLUM E	QUANTI TY
1	Sample: Extracted Silica Gel	3g	1
2	Reagents: Saline water	3ml	1
3	Culture: <i>A.niger</i> <i>S.cerevisiae</i>	0.1 ml 0.1 ml	1 1
4	Paper disc: Gentamicin (10 mg)		1
5	Glassware: St. MH agar butt St. Petri Plate St. Pipette St. Test tubes	25ml 4 10 6	2 2 2 2
	Miscellaneous: Disinfectant dish Cork borer Cotton Swab	5ml	1 1 1

PROTOCOL FOR EXTRACTION OF SILICA:

Disperse RHA in 1N HCl and stir for 2 hrs. Filter and wash the residue with water. Disperse residue in 1N NaOH and boil with continuous stirring. Filter and wash the residue with boiling water.

Cool the filtrate and titrate it with 1N H_2SO_4 until pH 7. Allow it gel for 24 hrs. Add water and crush the gel. Centrifuge at 2500 rpm. Discard the supernatant and repeat the washing. Dry at 50 for 2 hrs.

METHOD FOR EXTRACTION OF SILICA

Heat Treatment: The RHA obtained from burning paddy husk contains some product of incomplete combustion. To get complete pure burnt ash heat treatment is given. Sample was taken in a crucible and heated in an electrical oven for 700°C for 2 hours.



Heat Treatment

Grinding

Grinding: The grinding step to decrease mean particles size and increase specific surface area. For grinding RHA we use mortar pestle.

Acid washing: The aim of acid pretreatment is to improve the purity of silica products. It proves to be an effective way in substantially removing most of the metallic impurities and producing ash silica completely white in colour. 60 grams of RHA sample was dispersed in distilled water. 1N HCl was then added to this solution. Stirring is done for 2 hours to this dispersion. Next the ash was filtered using filter paper. The ash obtained was sent for silica extraction.



Acid washing

Silica Extraction: 500ml of 1N NaOH is used for the extraction, 60 gram ash and NaOH are put in a container at a temperature 90°C and atmospheric pressure with constant vigorous stirring at 900 rpm. The method of Kamath and Proctor (1998) was used for extraction of silica from RHA. After constant stirring the solution was filtered through ash less filter paper, the carbon residue was washed with 100 ml distilled water. The filtrates and washing were allowed to cool down to room temperature. The filtrate formed in this process is nothing but sodium silicate solution. The reaction occurred as follows:



Filtration: The solution is filtered through filter paper, and the carbon residue is washed with 100 ml of boiling water. The filtrate obtained contains sodium silicate solution which is a clear, transparent solution brownish yellow in colour and used for further process. The solid cake retained contains fly ash and other metallic impurities.



Filtration

Gel preparation

Gel preparation: The filtrate and washings were allowed to cool to room temperature and were titrated with 1N H_2SO_4 with constant stirring to pH. Silica gels started to precipitate

when the pH decreased to <10. The silica gels formed were aged for 18 hr. Distilled water (100 ml) was added to gels and then the gels were broken to make a slurry. Silica gel is produced by using various acids and different concentrations which is depicted in the above figure. Silica gel produced from IN H₂O₄ showed good results and has a higher specific area and took less time for gelification.



Gel preparation

Washing & Centrifugation: Distilled water is added to gels and then the gels are broken with glass rod to make slurry. Slurries are then centrifuged to remove the alkaline water;



Centrifugation

the clear supernatant is discarded. Purification of this silica for removal of sulphate impurities constitutes the third step of the process. For this successive demineralized water washings are given in the filter process itself. The conductivity of the effluent follows a decreasing trend owing to removal of sodium sulphate. Thus, conductivity can be used as the criteria to decide the number of washing's for obtaining silica of desired purity.

Drying: The gels are transferred into a beaker and again washed with ethanol, allowed to age for 2 hours so that the water gets displaced by the ethanol and dried at 50°C for 2 hr.. Ethanol is used so that the gel structure

does not get disrupted and shrinkage of the silica gel should be minimum.

PROTOCOL FOR ION EXCHANGE CHROMATOGRAPHY

Weigh 5 grams of extracted silica. Add 20 ml distilled water and stir well. Decant and add 1 ml HNO₃ (Keep it for 1 hour). Prepare a column of silica slurry in a burette. Take 20 ml aqueous solution of any cation dye in the column. For example, Malachite green. Observe characteristic colour over the column i.e. green. To elute exchanged colour, take 20 ml of 2M HCl and add to the column. Observe column losing colour because of ion exchange.

PROTOCOL FOR ANTI-MICROBIAL TESTS

AGAR CUP METHOD:

Inoculate 0.1 ml *S.aureus*, *E.coli*, *K.pneumoniae* culture to 20 ml cooled, melted MH agar butt mix and pour into sterile plates. Allow it to solidify. Make 4 wells in each plate using a sterile borer.

Transfer 0.1 ml of different dilution of extracted silica gel into each well of plates aseptically using pipettes. Incubate the plates at 37°C for 24 hrs. Observe the results after 24 hrs.

DISC DIFFUSION METHOD:

Dip a sterile cotton swab in the culture of the *S.aureus*, *E.coli*, *K.pneumoniae*; rotate the swap several times firmly on the inner wall of the tube to remove the excess of the culture.

Swap the isolate on the MH agar three times giving a period of 5 mins of absorption after each spreading. Allow the surface to dry for 5 mins before transferring the antibiotic discs and paper discs. The antibiotic discs are transferred on the inoculated MH agar plate aseptically using a sterile forcep. The disc must be in proper contact with the medium so that uniform diffusion of the antibiotic takes place. Incubate the plates at 37°C for 24 hrs. Observe the results after 24 hrs.

PROTOCOL FOR ANTI-FUNGAL TEST

Dip a sterile cotton swab in the culture of the *A.niger*, *S.cerevisiae*; rotate the swap several times firmly on the inner wall of the tube to remove the excess of the culture.

Swap the isolate on the MH agar three times giving a period of 5 mins of absorption after each spreading. Allow the surface to dry for 5 mins before transferring the antibiotic discs and paper discs. The antibiotic discs are transferred on the inoculated MH agar plate aseptically using a sterile forcep. The disc must be in proper contact with the medium so that uniform diffusion of the antibiotic takes place. Incubate the plates at 37°C for 24 hrs. Observe the results after 24 hrs.

RESULTS

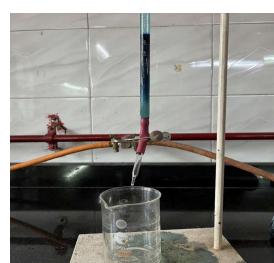
EXTRACTION OF SILICA GEL:



Silica gel is produced after calcination at 700°C for 2 hrs. The treatment using heat of RHA does not affect the structure of its silica. This study reveals that silica content is 93% and minimal mineral contaminants can be produced from RHA using simple chemical methods. The silica extracted from 60 g of RHA was 19.64%. It was possible to obtain high specific area silica from RHA after heat treatment and milling processing by applying this simple technique and it is possible to transform industrial residue in useful raw materials avoiding damage to the environment.

ION EXCHANGE CHROMATOGRAPHY:

The Ion Exchange Chromatography was performed by silica gel which was extracted by RHA. The cation dye i.e Malachite green was successfully separated by silica gel.



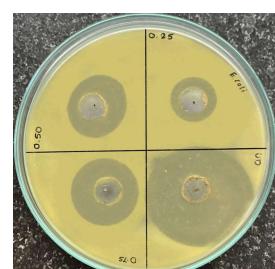
This indicates that extracted silica can be used for the chromatography or separation processes, also silica is highly purified. It can be used for separation of any type of charged molecule including amino acids, dyes, large proteins and small nucleotides.

ANTI-MICROBIAL TEST

Anti-microbial tests were performed by two methods i.e. Agar cup and Disc diffusion method

A. In Agar cup method:

1. *E.coli* was tested against different dilutions of extracted silica
 - For 0.25mg/ml zone of inhibition was 2.4 cm
 - For 0.50mg/ml zone of inhibition was 3 cm
 - For 0.75mg/ml zone of inhibition was 3.4 cm
 - For undiluted zone of inhibition was 3.7 cm



E.coli



S.aureus

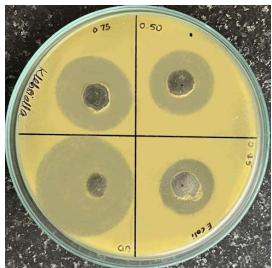
2. *S.aureus* was tested against different dilutions of extracted silica

- For 0.25mg/ml zone of inhibition was 1 cm
- For 0.50mg/ml zone of inhibition was 1.5 cm
- For 0.75mg/ml zone of inhibition was 2.3 cm
- For undiluted zone of inhibition was 2.8 cm

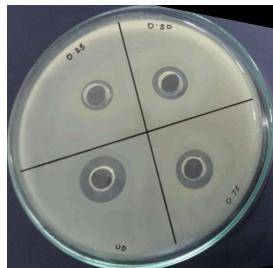
3. *K.pneumonia* tested against different dilutions of extracted silica

- For 0.25mg/ml zone of inhibition was 1.5 cm
- For 0.50mg/ml zone of inhibition was 1.8 cm

- For 0.75mg/ml zone of inhibition was 2.2 cm
- For undiluted zone of inhibition was 2.8 cm



K.pneumoniae



P.aeruginosa

4. *P.aeruginosa* tested against different dilutions of extracted silica

- For 0.25mg/ml zone of inhibition was 0.8 cm
- For 0.50mg/ml zone of inhibition was 1cm
- For 0.75mg/ml zone of inhibition was 1.1cm
- For undiluted zone of inhibition was 1.3 cm

B. In Disc diffusion method

1. *E.coli* was tested against gentamicin(10 mg) and paper disc(silica)
- Zone of inhibition for gentamicin(10 mg) was 2.5 cm
- Zone of inhibition for paper disc(silica) was 2.5 cm



E.coli

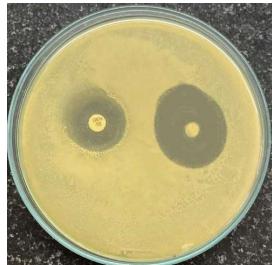


S.aureus

2. *S.aureus* was tested against gentamicin(10 mg) and paper disc(silica)

- Zone of inhibition for gentamicin(10 mg) was 2.3 cm
- Zone of inhibition for paper disc(silica) was 3 cm

3. *K.pneumonia* against gentamicin(10 mg) and paper disc(silica)
- Zone of inhibition for gentamicin(10 mg) was 1.2 cm
- Zone of inhibition for paper disc(silica) was 1.5 cm



K.pneumoniae



P.aeruginosa

4. *P.aeruginosa* against gentamicin(10 mg) and paper disc(silica)

- Zone of inhibition for gentamicin(10 mg) was 1.2 cm
- Zone of inhibition for paper disc(silica) was 1.5 cm

ANTI-FUNGAL TEST

Anti-fungal tests were performed by Disc diffusion method

1. *S.cervisiae* was tested against gentamicin(10 mg) and paper disc(silica)

- No zone of inhibition was observed for gentamicin(10 mg)
- Zone of inhibition for paper disc(silica) was 1.5 cm



S. cervisiae



A. niger

2. *A. niger* was tested against gentamicin(10 mg) and paper disc(silica)

- Improper zone of inhibition was observed.

CONCLUSION

Silica being inert, biodegradable and easily available from the waste rice husk serves as a very cheap column for the chromatography.

The porous nature of the silica from rice husk makes it more appropriate. Rice husk is abundantly available and it produces about 85 to 98% of silica. The purity of the silica produced from the rice husk is very high. The silica also showed anti-fungal and anti bacterial properties which further made it a suitable material for making columns in chromatography. The column has a very less chance of contamination. It is non toxic, however burning of rice husk produces a little bit of smoke and ash.

FUTURE PROSPECTS

- The silica produced from the rice husk is cheaper than the silica brought from the market, as it is produced from the agricultural rice husk waste.
- It has huge benefits such as it can be used as a chemical product in a chemistry laboratory.
- Extracted silica can be used to keep moisture free of optical instruments.
- Silica can be employed in the flower industry for drying and storage of flowers and seeds.
- It can also be used in chromatography techniques, food additives, humidity indicators and water filtration.
- Silica is used for diagnostic test strips, inhalation devices, syringes, drugs test kits and hospital sanitation kits.

REFERENCES

- Extraction Of Silica Gel From Rice Husk Ash For Promising Sustainable Industrialization: Statistical Analysis
Authors: Addis Lemessa Jembere
January 2019
DOI:10.18642/jmseat_7100122004
https://www.researchgate.net/publication/332703596_EXTRACTION_OF_SILOICA_GEL_FROM_RICE_HUSK_ASH_FOR_PROMISING_SUSTAINABLE_INDUSTRIALIZATION_STATISTICAL_ANALYSIS
- Preparation, Characterization and Antimicrobial Properties of Nanosized Silver-Containing Carbon/Silica Composites from Rice Husk Waste

Author: Felix Unglaube, Alexander Lammers, Dr. Carsten Robert Kreyenschulte, Prof. Dr. Michael Lalk, Dr. Esteban Mejía

First published: 13 December 2021
<https://doi.org/10.1002/open.202100239>

- A Simple Method for Production of Pure Silica from Rice Husk Ash Uruthira Kalapathy (Claflin University)
A. Proctor
John L Shultz (University of Arkansas)
July 2000
Bioresource Technology
73(3):257-262
DOI:10.1016/S0960-8524(99)00127-3