# Study Of Agar And Selection Of Suitable Agar Medium For Nitrate And Phosphate Reducing Bacteria

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Abstract: Water is wasted by many ways .Using water for washing cloth is one of the common way of wasting water. We know that nitrate and phosphate are main harmful content in the detergent. So it is necessary to treat wastewater generated after cloth washing and reuse it for various purposes. There are various methods and treatment available for removing nitrate and phosphate. Also different type of agar is also useful in wastewater treatment. Agar, more correctly known as agar-agar, has been used in the East for several hundred years and certainly since the seventeenth century. Agar is traditionally claimed to have been discovered by Tarazaemon Minoya in 1658 in Japan. Agar is useful in the various industries and also agar is very strong content. . In this paper we are study of different agar and selecting suitable agar medium for removing nitrate and phosphate reducing bacteria.

#### Keywords-Agar, Detergent, Nitrate Reducing, Phosphate Reducing, Treatment, Wastewater.

# I. INTRODUCTION

Agar is typically a strong gelling polysaccharide derived from red seaweeds and is characterized by its chemical repeat units of 3-6, anhydro L-Galactose. Agar also contain sulphate esters in low levels and some methoxy groups. Agar is eaten extensively in Asia has a flavoured jelly where the brittle texture is appreciated. However for western tases, brought up on gelatin, this is less acceptable. Agar is useful in the food industry as a gelling and thickening agent with particularly good properties in acidic dairy products where it is used as a stabilizer. Another key property of agar is the large hysteresis between the melting and setting temperatures which is unusual for a polysaccharide. One of the single biggest users of agar in the west is in the baking industry where the very high melting points of the agar gels make them particularly suitable to the baking process. Agar has good compatibility with sugar and can be used in very high sugar environments that would precipitate most other gums. Agar is often used to provide structure to high sugar systems such as doughnut icing.

So we can say that agar is very beneficial for other industry and it is harmless to ecosystem. For that same reason we study different agar and their use in wastewater. After study we selecting suitable agar for isolating nitrate and phosphate reducing bacteria.

## II. EASE OF USE

#### A. Avaibility of water.

In this paper we have studied different agar for using in detergent degrading bacteria. For the same reason we need wastewater generated after cloth washing. This wastewater is easily available in our daily household washing, Girls and boys Hostels, Hospital also in Dhobighat.

#### B. Avaibility of material

We are focusing low cost agar treatment that is easily available in laboratory. Also we have studied four different agar medium. These entire four agar are used for removing nitrate and phosphate.

## C. Avaibility of instruments

In this study we are using easily available instruments like incubator, Agar plate, Autoclave, Container, Easily available lab material(Test tubes, pipette)etc.

# **III. THEROTICAL CONTENT**

#### A. Study of agar:

#### I. Nitrate Agar:

Nitrate is the supply of nitrogen for several bacterium and fungi however it should be dampened to be utilized. The bacteria family called Enterobacteriaceae having ability to reduce nitrate to nitrite. Nonfermenters and alternative Nonfermenters and other miscellaneous gram-negative bacilli vary in their ability to reduce nitrates, some being capable of further reducing nitrite to nitrogen gas. The end product of reduction depends on the bacterial species. In 1962, Daubner reported that all **D**embers of the Enterobacteriaceae tested, with the exception of Erwinia species, reduced nitrate in 8 hours.

Table 1	Composition	of nitrate	agar."
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Ingredients	Gms/Litre	
Peptic digest of animal tissue	5.000	
Beef extract	3.000	
Potassium nitrate	1.000	
Agar	12.000	

Principle and Interpretation: Pure Culture Study of Bacteria of the Society of American Bacteriologist published formula for preparation of nitrate agar. Enterobacteriaceae family, Non-fermenters and other miscellaneous gram-negative bacilli fluctuate in their capacity to diminish nitrates is significant for separating and distinguishing different sort of microscopic organisms. Reduction of nitrate to nitrogen gas is done by some members of this group that process are called as denitrification.

First we could include 0.1 percent potassium nitrate substrate (KNO<sub>3)</sub> for breakdown nitrate.Particular Bacteria convert nitrate to nitrite and from nitrite to nitrogen gas.

For identifying changes we could include 0.5 ml sulphanilic acid and alpha-naphthylamine solution. By using red dizonium dye red violet colour will be developed that colours changes indicates that nitrate reduction to nitrite. If no colour changes we can conclude that nitrte is not reduced or further reduction to nitrogen gas has taken place. This can be identifying by adding little amount zinc dust.

#### **II. Pikovaskayas Agar:**

For the phosphate utilizing soil microorganism we are using Pikovaskaya agar. This is the standard agar for phosphate reducing microoraganism.

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Ingredients	Gms/Litre
Yeast extract	0.500
Dextrose	10.000
Calcium phosphate	5.000
Ammonium sulphate	0.500
Potassium Chloride	0.200
Magnesium sulphate	0.100
Manganese sulphate	0.0001
Ferrous sulphate	0.0001
Agar	15.000

# Table.2. Composition of Pikovaskava agar.<sup>5</sup>

#### **Principle and Interpretation:**

Both organic and inorganic forms of Phosphate exists in soil. rich organic sources of phosphorus are Organic matter derived from dead and decaying plant debris . only in the

free available form of phosphorus plants are able to utilize them from soil. For plant roots and soil microorganisms Soil phosphates are easily available . Therefore, phosphatereducing soil organisms play a role in part for correcting phosphorus deficiency of crops (1). Pikovskayas Agar was modified by Sundara Rao and Sinha (2) for detection of phosphate-utilizing bacteria from soil.

medium provides nitrogen and other nutrients necessary to support bacterial growth in the form of Yeast extract. energy source provides in the form of Dextrose. The growth of organisms is enhanced by different salts and yeast extract. Phosphate-utilizing bacteria will grow on the medium and form a clear zone around the colony, formed due to phosphate utilization in the vicinity of the colony.

#### **IV. RESULT**

1) Nitrate agar having ability to give maximum positive results for isolation of nitrate reducing bacteria.

2) The ability to reduce nitrate is valuable for differentiating and identifying various types of bacteria especially Enterobacteriaceae family.

3) Pikovaskaya agar having ability to give maximum positive results for isolation of Phosphate reducing bacteria. 4) In this study we found out suitable agar for isolation of nitrate and phosphate reducing bacteria for removal of phosphate and nitrate from wastewater sample containing detergent

# V. CONCLUSION

We can conclude that this methods are particular and the most efficient methods for maintaining clean environment and clean water. We can also conclude that Biological methods are ecofriendly and easily applicable in environment. Agar is easily biodegradable, easy to store. It is safe and harmless for use. This method is non carcinogenic.

We are also concluding that this biological method is suitable for removal of Nitrate and phosphate from wastewater generated after cloth washing. We conclude that this is low cost method because bacteria having sustainable growth quality.

After the study we may say that nitrate agar and pikovaskaya agar are most effective medium for next study.

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