Bioremediation of Rice Mill Effluent Using *Pseudomonas* spp

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Abstract
Bioremediation has been recognized as a environment friendly and less expensive method which involves the natural processes resulting in the efficient conversion of hazardous compounds into innocuous products. Recently microbial bioremediation has emerged as an alternative technique to such traditional chemical treatments. Rice mills consume large quantities of water for various processes and discharge equally large volumes of waste waters containing variety of pollutants and heavy metals. In the present study attempt has been made to bring about the bioremediation of rice mill effluent collected from Onyx rice mill along Bida-Baddegi road,Bida Niger state. Pseudomonasspp were isolated from the site of contamination and were employed for this bioremedial study. The bacteria spp were able to bioremediate the rice mill effluent after monitoring the rate of absorbance and concentration of reduction potential in the effluent sample using a spectrophotometer. Maximum degrading potential observed at different concentration of the rice mill effluent i.e absorbance at 20,30,50-90% (0.310±0.001,0.314±0.002,0.543±0.002,0.582±0.002,0.589±0.002Au)respectively.From this study it can be inferred that Pseudomonas species have the potentials to clean up environment polluted with effluents of rice mill.

Keywords: *Bioremediation RicemillPseudomonas Effluent Pollutants*

INTRODUCTION

Rice is the seed of the grass species *Oryza sativa* (Asian rice) or *Oryzaglaberrima* (African rice). As a cereal grain, it is the most widely consume staple food for a large part of the world's human population, especially in Asia. It is the agricultural commodity with the third-highest worldwide production (rice, 741.5 million tonnes in 2014), after sugarcane (1.9 billion tonnes) and maize (1.0 billion tonnes). (Food and Agriculture Organization, Corporate Statistical Database (FAOSTAT), 2019)

Since sizable portions of sugarcane and maize crops are used for purposes other than human consumption, rice is the most important grain with regard to human nutrition and caloric intake, providing more than one-fifth of the calories consumed worldwide by humans. (Smith,2018).There are many varieties of rice and culinary preferences tend to vary regionally.Rice, a monocot, is normally grown as an annual plant , although in tropical areas it can survive as a perennial and can

produce a ratoon crop for up to 30 years. (International Rice Research Institute (IRRI),2019).

Rice cultivation is well-suited to countries and regions with low labor costs and high rainfall, as it is labor-intensive to cultivate and requires ample water. However, rice can be grown practically anywhere, even on a steep hill or mountain area with the use of water-controlling terrace systems. Although its parent species are native to Asia and certain parts of Africa, centuries of trade and exportation have made it commonplace in many cultures worldwide.The traditional method for cultivating rice is flooding the fields while, or after, setting the young seedlings. This simple method requires sound planning and servicing of the water damming and channeling, but reduces the growth of less robust weed and pest plants that have no submerged growth state, and deters vermin. While flooding is not mandatory for the cultivation of rice, all other methods of irrigation require higher effort in weed and pest control during growth periods and a different approach for fertilizing the soil.Rice mill generate waste mainly in liquid, solid and gaseous form,husk and ask constitute the main bulk of solid waste,however husk is reused in the mill for boiling water so residue left after combustion is ash. Gaseous emission left is mainly SO_2,CO_2,and NO_3.Beside these, rice mill generate waste from the following operations; parboiling wastewater, paddy soaked wastewater, and boiler blown down.The liquid waste from this operation ultimately passes through a common drain to the outside of the mill boundaries and this is commonly called as combined effluent or combined wastewater.In recent years considerable attention has been paid to industrial waste which are usually released into different water course. Increasing environmental awareness for various rice mill industries to install or set up appropriate waste wastewater treatment technology. As the number of mill has grown, adequate waste water management has become very much essential.To pit it simple rice mill effluent has an adverse effect on natural environment.Rice mill often release huge volume of underwater which often contain huge volume of under water and other by product when processing rice.The wastewater often contains toxic inorganic and organic contaminants which causes environmental damage when released.Huge number of rice mills engaged in rice milling which is the process of removing husk from paddy to produce edible rice. In the rice mills, the water is mainly used to soak the rice and they release huge volumes of wastewater and other by-products when processing paddy rice. The rice mill effluent coming from various rice mill operations often contains high concentration of toxic organic and inorganic matter which causes significant environmental damage when released.

Rice Milling

Rice milling is the process of removing the husk and part of the bran from paddy in order to produce edible rice but also carries out many other essential function such as procurement, drying,storage, quality control and utilization of by- product .parboiled rice production generally requires large amount of water for soaking of the paddy. This water of not properly treated could result in water pollution and odour nuisance to residents. Water pollution can be caused by high levels of organic materials present in water.Effluent produced during cleaning of the equipment may cause water pollution through insufficient treatment of the effluent .The drastic environmental changes mainly due to rapid industrialization have emerged as major challenges over the world. It is very much necessary to increasing awareness of the fact that clean environment is necessary for

smooth living and better health of human begins.Primary milling of rice is most important activity in food grains. These grains are grown and used in some parts of Nigeria.Due to industrialization and global competition market trends it has emerged as major industrial activity in small medium sector to cater the needs of increasing population. There are huge number of mills engaged in processing of rice and are spread over in almost all state across the country due to increasing trends. It is essential that the 80% of world population uses rice as major sources of calories. Wastewaters coming from rice mill operations contains high concentration of organic and inorganic substances causing significant polluting phenomenon. In order to sustain our global water supply many environment directives,regulations and legislatives has been issued in order to define quality standards for water.The high chemical oxygen demand suspended solids,conductivity,salinity and total dissolved solid still pose an economical problem for the industries since these have been employed as major parameter. This study is aimed at bioremediatingrice mill effluent using P*seudomonas* species

MATERIALS AND METHODS

Sample Collection

The rice mill effluent was collected from Onyx rice mill Bida,Niger State,Nigeria. The rice mill effluent collected was analysed in in the laboratory for BOD ,COD, Nitrate, phosphate, sulphate, turbidity,total waste and colour were determined using standard method (APHA,2019).

PHYSICOCHEMICAL ANALYSIS

Determination of Colour Intensity

The colour intensity of the sample was determined with the aid of a lovibond , comparator by matching the colour of the sample with standard.(APHA,2019;Ademoroti,2021).

Determination of Total Solid(Ts)

A clean dish of suitable size was dried at 102-105°C in an oven to a constant weight.100-250ml of thoroughly mixed effluent sample was accurately pipette into a dish weight and evaporated by dryness on a steambath.The residue was dried in an oven for about 1hour at 103-105°c and reweighed after cooling to room temperature.The cooling was done until the weight of the dish plus residue was constant to within 0.05mg.The weight of the dish was subtracted to obtain the weight of the total solids (APHA,2019,Ademoroti,2021).

Determination of Dissolved Solid (D's)

Dissolved solid were obtained by difference between total solid and suspended solid(APHA,2019;Ademoroti,2021).

Determination of Chemical Oxygen Demand(COD)

The untreated sample of the effluent was first analyzed for the BOD, immediately after collection. The biological treated sample was also analyzed for COD as earlier reported(APHA,2019;Asamodu,2020). Nitrate, sulphate and phosphate level were determined according to Ajao(2021).

Preparation of Media(MacConkey agar)

Some grams of macConkey agar was dissolved in distilled water and was Heated to boiling to dissolve the medium completely.It was then Sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes,and Cool to 45-50°C.It was mixed properly and was dispensed into sterile Petri plates.

Isolation and Identification of*Pseudomonas* and *Bacillus* species

From the rice mill effluent collected, 1ml was taken and it was serially diluted with distilled water. The serial dilution was done up to 10-5.From each dilution,1ul of sample was spread on MacConkey agar plate.The plates were incubated at 37°c for 24hours.the colonies appeared on MacConkey agar plate were subcultured and identified using biochemical and morphological test and stored in agar slant.

Effluent Concentration

The rice mill effluent was prepared in different concentration ranging from 10-100% using distilled water into sterilized caped bottled.

Bioremediation Process

The bioremediation of rice mill effluent was examined under laboratory conditions by the introduction of the isolated *Pseudomonas* species in to prepared concentration of the rice mill effluent. Nutrient was added to hasten bioremediation process. The rate of degradation was examined for 60 days at appropriate temperature after which the absorbance and concentration was determined using a spectrophotometer.

RESULT AND DISCUSSIONS

Result

Physicochemical and Microbiological Examination of the Sample

Apparently,the effluent sample which was collected from Onyx rice mill in Bida Niger state,Nigeria was of disagreeable smell and temperature of about 38°c. The PH of the effluent was about 4.8 ,total solid in the effluent was 3.79g/L. The COD of the effluent was about 23338mg/L than BOD of 1559mg/L. The physiochemical parameter of the effluent sample was compared with environmental standard and shown in table 1.

Characterization and Identification of the Strain

At the stage of preliminary screening the selected bacterial were subjected to morphological, physiologically and biochemical characteristics. The colony characteristics of the selected bacterial on MacConkay agar and microscopic features are presented in table 2.

In table 3 is shown the absorbance of Bacillus and Pseudomonas species on rice mill effluent after treatment.

At 100% concentration,the absorbance on Pseudomonas was highest (0.251A.U)and significant difference ($p<0.05$) than the absorbance on Bacillus species (0.242A.u). A similar trend was observed at 40% concentration (0.454 and 0.332:Pseudomonas vs Bacilli). Also shown in table 3: the absorbance of rice mill effluent on Bacillus species was observes to be higher (0.310Au) and this differ($p<0.05$) from the absorbance of rice mill effluent on Pseudomonas specie(0.260 Au).A similar trend was observes at 30(0.314 vs 0.263),50(0.543 vs 0.463),60(0.582 vs 0.565),70(0.589 vs 0.441),80(0.644 vs 0.536) and 90%(0.698 vs 0.554 Au).Furthermore the control 100% did not show any significant difference ($p>0.05$) among the two test organism (Table 3).

Table 4. Is shown the Concentration of Bacillus and Pseudomonas species on rice mill effluent after treatment .

At 10% concentration of effluent,the concentration on Pseudomonas specie was highest at (250.000mg/L) and significantly different ($p<0.05$)than the concentration on Bacillus species (243.000mg/L). Similar trend was observed at 40% concentration (452.000mg/l and 341.667mg/l: Pseudomonas vs Bacillus). Also shown in table 4,the concentration of rice mill effluent on Baccilus species was observed to be higher at 20%. (309.667mg/l) and this differd ($p<0.05$) from the concentration of rice mill effluent on Pseudomonas species (257.000mg/l). A similar trend was observed at 30(323.000 vs 264.667mg/L),50(644.333 vs 462.333mg/l)60(644.333 vs 564.000mg/l),70(583.333 vs 534.333),80(647.000 vs 536.333mg/l). Furthermore the control did not show any significant difference ($p<0.05$) among the two test organism(Table 4).

Table 1.Pysicochemical characteristics of rice mill effluent

Parameters	Value
Odour	foul
Colour	turbid
PH	4.8
Temperature	38°c
BOD	1559(mg/L)

COD 2338(mg/L)

Total solid 3.79(g/L)

Mg 0.015(g/m)

K 2.66(g/m)

Ca 0.015(g/L)

Table 2. Showing Biochemical and Morphological characteristics of bacterial isolated from rice mill effluent

Biochemical characteristics	organism.(Bacillus spp)	organisms (pseudomonas spp)
Catalase.	+	+
Indole.	-	-
Gram staining.	+	-
Oxidase	+	+
Shape.	Rod	Rod
Spore	+	-
Motility.	Motile	motile
Coagulase	-	-
Flagella	Flagellated	Flagellated

Table 3.Showing result for absorbance of effluent after treatment with Bacillus and Pseudomonas species after treatment for 60days

	Effluent concentration (%)								
Organism	10	20	30	40	50	60	70	80	90
Bacillus	0.232	0.310	0.314	0.332	0.543	0.582	0.589	0.644	

0.698 , ± ±
± ± ± ± ± ± ±

0.001 0.001 0.002 0.001 0.002 0.002. 0.002 0.023 0.002 Pseudo

monas 0.251 0.260 0.263. 0.454. 0.463 0.565 0.441 0.536 0.554

± ± ± ± ± ± ± ± ±

0.000 0.001 0.001 0.002 0.002 0.003. 0.002 0.002 0.005

1.Each data is the mean standard of triplicate determination

2. Different letters within the same column are significantly different ($p<0.05$)

Effluent Concentration(%)

Organism 10 20 30 40 50 60 70 80 90

Bacillus 243.000 309.667 323.000 341.667 644.333 644.333 583.333 647.000 699.667

± ± ± ± ± ± ± ± ±

0.577 0.333 0.577 1.201 2.333 2.603 1.764 647.000 4.842

Species

Pseudomonas 250.000 257.000 264.667 452.000. 462.333 564.000 534.333. 536.333 554.000

± ± ± ± ± ± ± ± ±

Species.0.577 0.577 0.333. 1.528. 1.202 2.309. 2.3333. 3.180. 2.646

Discussion

The table1, represent the result obtained in the initial characterization of the sample of the effluent from onyx rice mill Bida Niger state,Nigeria. The result indicate the mean value of COD,BOD,TS respectively .heavy metals were also higher than the permissible limit, if the effluent is not treated before being discharged into the receiving river or soil it can pose ecological threat.A high BOD value shows that the effluent have higher oxygen demanding waste(Kumar,2019) which causes the depletion of Demanding oxygen which is a fundamental requirement for aquatic life the higher

value of COD gives valuable information about the pollution potential of the rice mill industrial effluent.

The colloidal and suspended impurities causes turbidity in the receiving streams and reduce light penetration in the receiving streams and ultimately decreases the photosynthesis (Sofianostress et al.,2020).Total solid determined in this study has great implication in the biological and physical waste water treatment process (Srivasta and Sinba, 2021:Tobata et al.,2019:Ashish and Yogendra 2019: Ajao et al.,2021).

The dissolved minerals may increase salinity of the water and this may render it unfit for irrigation or consumption.Toxic characteristics such as chromium and sulphites may destroy fishes and Microorganisms responsible for self-purification of water impurities can cause depletion of dissolved oxygen content of water.water contaminated with mettalic effluent can causes several health problems,this can interfere with enzymes activities and function of red blood cells.it can affect nerves and brain at low concentration.(Ezeronye et al.,2018).The appearance of rice mill effluent was found to be brown and turbid,the turbidity is due to the shape,size and refractive index of the particulate impurities like timely divided organic and inorganic matter and coloured compounds present in the water sample. It changes the colour of the water,retard the penetration of sunlight with decrease in photosynthetic activity and depletion of oxygen content and make the water unsuitable for use.

Odour- The smell or odour of the rice mill effluent was found to be unpleasant or foul, the offensives odour may be due to the volatile substance associated with organic matter and anaerobic decomposition by living organism, principally microorganisms. The offensive odour impairs the water quality and causes nausea and vomiting.

temperature- Temperature is the measure of hotness or coldness of a substance. The measurement of temperature in water is important basically for its effects on the biochemical reactions in the living organism. It is also important in the determination of PH,conductivity and saturation level of gases in water .in this present study, the average temperature of the rice mill effluent was about 38.0°C

PH- PH is the measurement of hydrogen ion concentration and it indicates instantaneously the intensity of acidity or alkalinity in water of effluent .it effect many chemical reactions and biological system functions only in relatively narrow PH.

Based on the result of biochemical test parameter(table2)the isolates were identified as Bacillus and Pseudomonas species, the identification was done by comparing the experimental result of biochemical test parameter with the limited description in Bergey's Manual of Determinative Bacteriology(8th Edn.)

Degredation of of rice mill effluet by the isolates:

In the present investigation, isolates were tested for their ability to degrade different concentrations

(10-90%) of the effluent after 60 days of incubation period Satisfactory degradation by these bacteria were seen, degradation pattern of each concentration of the effluent are given in table 3 and 4 respectively. The spectrophotometry concentration and absorbance used to determine the rate of degradationshows the reduction in the level of heavy metals, BOD,COD, TSS etc, present in the effluent sample. The Absorbance recorded at different concentration of the effluent as shown in tables 3 shows that the rate of degradation increases as the effluent concentration Increases, this is in agreement with experiment carried out by(Malik et al 2007)on bioremediation of Sellarice mill effluent

Also shown in table 3,it was recorded that effluent treated with Pseudomonas specie at 10 and 40% concentration has higher absorbance in comparison to that treated with bacillus at the same concentration I.e at 10 and 40%(0.251,Au) compare to the absorbance on effluent treated with Bacillus I.e(0.242Au). The same trend was recorded in table 4 for measured Concentration (mg/l) Similar degradation potential of Bacillus and Pseudomonas species was reported previously by(Sharnaik and Kaneker, 2019; Mihir et al., 2022; Sukumar et al., 2021; Wang et al., 2019). Many bacteria have been reported to be involved in effluent degradation (Uddin et al., 2020). Further investigation is needed to understand the degrading capacity of these bacterial.

CONCLUSION AND RECOMENDATIONS

CONCLUSION

Bioremediationserve as a very effective method of removing poisonous contaminants and heavy metals from industrial effluent. It serves as a cheap and easy means of water treatment which leaves behind non-toxic by product since it involves living organism with the ability to transform toxic matter in their normal biological activities to yield non-toxic by- products.

Based on this research work, it is clear that bioremediation of rice mill effluents by bacteria is an effective method for treating rice mill effluent and can be substitute for conventional remediation process.

The rate of degradation of effluent water containing heavy metals is best done by introduction of microorganisms like *Pseudomonas* and *Bacillus species* and some nutrient.

RECOMENDATIONS

Based on this project work, the following recommendations were made:

- Careless disposal of Industrial waste without pretreatment should be discouraged
- Imposition of direct charges on industrial effluent by regulatory agencies as well as continuous monitoring and surveillance is imperative in order to ensure the protection of water resources from further degradation

- Further detailed study is needed to optimize process parameters for bioremediation of rice mill effluents using these bacterial isolates.

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