

## BIODEGRADATION OF RICE MILL EFFLUENT BY IMMOBILISED *PSEUDOMONAS* SP. CELLS

R. Manogari , D. Daniel , A. Krastanov

**Abstract:** Rice milling is the process of removing the husk and part of the bran from paddy in order to produce edible rice. Rice production, especially parboiled rice production, generally requires a large amount of water for soaking the paddy. This water, if not properly treated, could give rise to water pollution. The limitations in the physico-chemical methods make the biological methods favourable alternatives for the removal of pollutants from the industrial effluents. A bacterial strain was isolated and identified as *Pseudomonas* sp. through morphological and biochemical tests. Preliminary experiments with *Pseudomonas* sp. were carried out, using free and immobilised cells. Experiments in a packed bed system using immobilised cells indicated a 86.44 % reduction in the COD and a 55.34 % reduction in the BOD after 24 hours. The electrical conductivity, salinity and total dissolved solids were found to decrease to a considerable extent. Studies with varying cell loading showed that a cell loading of 1.7 – 2.2 mg cells per bead was capable of improving the quality of the rice mill effluent successfully.

**Key words:** biodegradation, rice mill effluent, immobilised cells, *Pseudomonas* sp.

### 1. INTRODUCTION

Wastewaters coming from different industrial operations contain high concentration of organic and inorganic substances causing significant polluting phenomena. In order to sustain our global water supply, many environmental operation programs have been established to address pollution issues. Numerous environmental directives, regulations and legislations have been issued in order to define quality standards for water. The high chemical oxygen demand, suspended solids, conductivity, salinity, and total dissolved solids still pose an economical problem for the industries since these have been employed as major parameters for effluent discharge. Various physico-chemical techniques have been studied for their applicability to the treatment of wastewaters (Alkhatim et al., 1998; Cassano et al., 2001; Song et al., 2004; Murugananthan et al., 2004; Badani et al., 2005; Ahmed et al., 2005; Rodrigues et al., 2007).

The limitations in the physico-chemical methods make the biological methods a favorable alternative for the removal of pollutants in the industrial effluents. The process of biodegradation is a well-established and powerful technique for treating domestic and industrial effluents. Microbial populations have an amazing and extensive capacity to degrade a variety of organic compounds (Atlas and Bartha, 1998). A number of studies are available on biodegradation of industrial effluents (Nisha 2002, Noorjahan et al., 2005; and Adekunle and Oluyode, 2005)

Wesenberg et al., (2003) have reviewed and summarized the prospective white rot fungi,

and their enzyme for the treatment of industrial effluents and Pearce et al., (2003) reviewed the potential of whole bacterial cells to metabolize the azo dyes present in colored aqueous wastewater from the textile industry.

Rice milling is the process of removing the husk and part of the bran from paddy in order to produce edible rice. Rice milling industry does not only mill rice but also carries out many other essential functions, such as procurement, drying, storage, quality control, and utilization of by-products. Parboiled rice production generally requires large amount of water for soaking of the paddy. This water if not properly treated could result in water pollutions and odour nuisance to residents. Water pollution can be caused by high levels of organic material present in wastewater. Effluent produced during cleaning of the equipment may cause water pollution through insufficient treatment of the effluent.

Literature reports indicate that biodegradation involving microorganism is a suitable process for wastewater treatment. Hence, in the present study, an attempt has been made to develop a process for biodegradation of rice mill effluents using microorganisms isolated in a laboratory. Based on the above discussions the following objectives were formulated for the present work: Isolation and characterization of bacteria for effluent degradation; Characterization of rice mill effluents; Study of rice mill effluent degradation using free and immobilized cells; Development of a packed bed system for the effluent treatment.

## 2. MATERIALS AND METHODS

### 2.1. Effluent Sample

M/s Agro Machinery and Consultancy Private Limited, Cochin (India) provided the rice mill effluent. It was stored in cold room at 4°C.

### 2.2. Media

For the isolation of the microorganisms from the effluent, Nutrient agar and MacConkey and Eosin methylene blue medium was prepared. The Nutrient agar medium had the following composition (g/l): peptone 5.0, beef extract 3.0, sodium chloride 5.0, and agar 15.0. The pH of the medium was 7.0, prior to autoclaving (120 °C for 20 minutes). MacConkey agar medium contained the following ingredients (g/l): peptic digest of animal tissue 20.0, lactose 10.0, bile salts 5.0, sodium chloride 5.0, neutral red 0.07 and agar – 15.0. The pH of the medium was 7.5, prior to autoclaving (120°C for 20 minutes). Eosin methylene blue medium contained the following ingredients (g/l): peptone 10.0, lactose 10.0, dipotassium hydrogen phosphate 2.0, eosin 0.4, methylene blue 0.065 and agar 13.5. The pH of the medium was 7.0, prior to autoclaving (120 °C for 20 minutes).

For the isolation of the *Pseudomonas* sp., King's B medium was used. The medium contained the following ingredients per litre: peptone – 20 g; glycerol – 10 g; dipotassium hydrogen phosphate – 1.5 g; magnesium sulphate – 1.5 g and agar – 15 g. The pH of the medium was 7.0, prior to autoclaving (120 °C for 20 minutes).

The gelatin liquefaction (hydrolysis) medium had the following composition (g/l): peptone 5.0, beef extract 3.0, gelatin 120.0. The initial pH of the medium was adjusted to 6.8.

### 2.3. Isolation of the microorganism and characterization of the isolated strain

After solidification of the nutrient agar medium, 0.1 ml of the given effluent was spread on to the agar medium and incubated at 37°C for 48 hours. The procedure was repeated with EMB, MacConkey, King's B medium. The isolate was identified as *Pseudomonas* sp. based on the morphological and biochemical test results – gram staining, catalase test, oxidase test, gelatin hydrolase test.

### 2.4. Growth of Culture in Shake Flasks

Cells from previously maintained agar slants were suspended in 10 ml sterile distilled

water. The cell suspension was then shaken thoroughly to break up any aggregates and was used as the inoculum of 100 ml sterile medium in Erlenmeyer flasks. The suspension cultures were grown for 48 hours at 37°C on a rotary shaker. 10 ml of broth culture was added to 100 ml of sterilized minimal medium. The flasks were kept on the shaker for 72 hours. Samples were taken at regular intervals. The samples were filtered through filter paper; the paper was kept in an oven at 80°C for 24hrs. The growth was determined in terms of dry cell weight.

### 2.5. Immobilization of the Cells

The cells from shake flask were used for immobilization in alginate beads. Fully-grown cells were separated by centrifuging at 5000 rpm for 15 minutes. The separated cells were washed repeatedly with sterile water to remove the soluble components. The cell suspension was then added to 2 % (w/v) sodium alginate solution. The resulting mixture was then dropped into 20 % (w/v) calcium chloride solution by using a peristaltic pump. The drops of sodium alginate solution gelled into 5.0 mm diameter spheres upon contact with the calcium chloride solution. The immobilized cells were incubated at room temperature (30 °C) for 2 hours and were stored at 4°C for further use.

### 2.6. Batch degradation using free and immobilized cells in shake flasks

Batch studies for the degradation of wastewater were conducted in Erlenmeyer flasks containing 100 ml of effluent and 10 ml of freely suspended culture volume. The flasks were agitated on a shaker at room temperature for 72 hours. The effect of the culture on the pH, temperature, salinity, conductivity, total dissolved solids, turbidity, BOD, DO, and COD of the effluent was determined.

Batch studies for the degradation of wastewater were conducted in Erlenmeyer flasks containing 100 ml of effluents and 100 beads containing the immobilized cells were added to these flasks and agitated on shaker at room temperature for 72 hours. The effect of the immobilized cells on the pH, temperature, conductivity, salinity, total dissolved solids, turbidity, DO, BOD, and COD of the effluent was determined.

### 2.7. Estimation of dissolved oxygen

One litre of dilution water was brought to 20 °C using an organic free filtered air. To this water 1 ml of each solution: phosphate buffer, magnesium sulphate, calcium chloride, and ferric chloride were added. 10 ml of the effluent was taken and 290 ml of dilution water was added to a reagent bottle with 2 ml of manganous sulphate and 2 ml of alkali iodide – azide solution. The bottle was stoppered and its contents were thoroughly mixed. The formed precipitate was allowed to settle down. 2 ml of sulphuric acid was added and the contents were shaken well. 100 ml of the sample was taken in a conical flask and 3-4 drops of starch solution were added. The developed blue color was titrated against sodium thiosulphate solution from a burette. From the end point the dissolved oxygen (mg/l) was calculated.

### 2.8. Estimation of biochemical oxygen demand

One litre of dilution water was brought to 20°C using an organic free filtered air. To this water was added 1 ml of each solution: phosphate buffer, magnesium sulphate, calcium chloride, and ferric chloride. 10 ml of effluent and 290 ml of dilution water were added to the BOD bottle. Initial dissolved oxygen was measured using the procedure outlined in the previous section. Another BOD bottle was filled with 10 ml of effluent and 290 ml of dilution water. The bottle was incubated at 20°C in the BOD incubator for 5 days. After incubation, the final dissolved oxygen was measured and BOD was then computed.

### 2.9. Estimation of chemical oxygen demand

10 ml of sample were mixed with 20 ml of potassium dichromate, 30 ml of silver sulphate – sulphuric acid mixture and 0.5 gm of mercuric sulphate in a COD reaction vessel. The reaction vessel was inserted into the heating block, which was preheated to 150°C. Air condenser was fixed on the reaction vessel and the contents were refluxed for 2 hours and then cooled. 80 ml distilled water were added and the excess potassium dichromate present was titrated against FAS using Ferroin indicator. Blank using distilled water was carried out and COD of the sample was calculated.

### 2.10. Measurement of Other Parameters

Electrical conductivity, salinity, total dissolved solids, and temperature were determined using a Water quality analyzer (Systronics, Ahmedabad). The instrument measured the conductivity, estimated total dissolved solids and salinity of the solution under test using a conductivity cell. Calibration of the instrument was carried out with standard potassium chloride solution. The temperature was measured using a PT 100 sensor. The PT 100 and the cell were dipped into a sample-containing beaker and an appropriate mode was chosen. The equipment showed the cell constant and the value of the parameter selected for measurement directly.

### 2.11. Turbidity

Turbidity was measured using a nephelometric turbidity meter. The instrument was calibrated using standard reference suspension (Formazin polymer). Appropriate range was selected (1000 NTU); the sample-containing cell was inserted into the cell holder and covered with light shield. The display directly showed the turbidity in NTU.

### 2.12. Packed bed reactor

A glass column (260mm x 20mm) was used for the biodegradation studies. The substrate was pumped from the bottom of the reactor. The bottom of the reactor was equipped with cotton wool for the uniform distribution of substrate. A side connection was provided at the top of the column for withdrawal of samples when required. Rice mill effluents were taken in a conical flask placed on a magnetic stirrer. After stirring, the mixture was fed to the reactor, from the bottom, using a silicone tube. A constant flow rate of substrate was maintained with the help of a peristaltic pump. At minimum substrate flow rate the contact time between the substrate and the catalyst bed was approximately 45-60 minutes. Continuous aeration was provided at a minimum level to the effluent, using an aerator pump. After the contact time, samples were withdrawn from the reactor at different time intervals and analyzed for the characteristics. Four experimental runs were carried out using different cell loading rates.

### 3. RESULT AND DISCUSSION

#### 3.1. Identification of the strain

The strain was isolated using the spread plate method and was identified as *Pseudomonas* sp. It was characterized using biochemical and morphological tests. In the nutrient agar plate, large, opaque, irregular colonies were observed. These were aerobic, nonspore forming, motile, gram-negative bacteria. A fluorescent green (Pyoverdine) color pigment was produced in nutrient agar medium. Non-fermenting colonies were observed on MacConkey agar plates. King's B medium was used for the selective isolation of *Pseudomonas* sp. The strain was tested positive for gelatin liquefaction test, catalase test, oxidase test and produced earthy smell (ash) during growth.

The population growth was studied by analyzing the growth curve of the culture. The resulting curve had four distinct phases. After the lag phase, the exponential phase started from the 15<sup>th</sup> hour with the stationary phase maintained at 30 to 48<sup>th</sup> hour. The culture exhibited a balanced growth in this phase. This was followed by a death phase due to nutrient depletion and generation of wastes in culture medium.

#### 3.2. Biodegradation using immobilized cells in shake flasks

Since the overall aim of the present work was to develop a process for treatment of rice mill effluents with the isolated strain, immobilized cells were used for the biodegradation studies. The variations in the characteristics of raw rice mill wastewater treated using a cell suspension and immobilized *Pseudomonas* sp. cells were studied. The electrical conductivity decreased by 9.77 % in the first 48 hours of treatment. After 72 hours the reduction was only 5.71 %. Salinity was found to decrease to 7.07 mS after 72 hours. The percentage reduction in the total dissolved solids was similar to that of the reduction in electrical conductivity (9.86 %). The turbidity was found to increase by 6.84% in comparison to that of the raw effluent. There was a marked decrease in the values for biological oxygen demand and the chemical oxygen demand which were found to reduce by 31.25 % and 22.5 %, respectively. However, the dissolved oxygen showed a considerable de-

crease at the end of 72 hours of treatment with the immobilized cells (10.52 %).

#### 3.3. Biodegradation using immobilized cells in a packed bed reactor

Experiments were carried out in a packed bed reactor at different cell loadings. In the first reactor study 0.9 mg of cells per bead was used. The characteristics of the raw effluent and the one treated with the immobilized cells are depicted in Table 1. Electrical conductivity was 11.4 mS in the raw effluent and it decreased to 2.37 mS in 17<sup>th</sup> hour itself. An initial salinity of 12.1 ppt in raw effluent was found to decrease 2.84 ppt in the 17<sup>th</sup> hour. The total dissolved solids were 4.64 ppt in the raw effluent and were reduced to 1.09 ppt in 17<sup>th</sup> hour. The turbidity of the effluent sample was observed to reduce from 431 NTU to 276 NTU in 17<sup>th</sup> hour. The chemical oxygen demand decreased by 66.85 %. Dissolved oxygen was recorded as 7.8 mg/l in the raw effluent, which further reduced to 6.2 mg/l in 17<sup>th</sup> hour. The biochemical oxygen demand reduced with 55.5% by the end of 17 hours.

From the above experiments is evident that the cells immobilized in a packed bed system offer better treatment as compared to the immobilized cells in a suspension culture. Therefore, further experiments were carried out to find the effect of different cell loading on the characteristics of the treated effluent. A second reactor involving 0.8 mg cells per bead was used for the degradation studies. It was found that the electrical conductivity, salinity and total dissolved solids reduction was similar to that obtained with the previous cell loading. The turbidity of the effluent was reduced by 10.25 % in the lower cell loading. The percentage decrease in COD, DO and BOD were determined as 56.08 %, 16.21 % and 3.84 % respectively. From the results is evident that with the decrease in cell loading, there is no appreciable improvement in the quality of the effluent.

In reactor study 3 was used a cell loading of 1.7 mg per bead. The characteristics of the raw effluent after treatment with the immobilized in a packed bed reactor cells for 24 hours are depicted in Table 1. The electrical conductivity decreased by 81.8 %. Salinity decreased by 80.81%. Total dissolved solids decreased by 81.02 %. The turbid-

ity of the effluent sample was observed to reduce from 430 NTU to 258 NTU. The percentage decrease in COD, DO, BOD was found to be 70.40 %, 16.66 % and 25.38 % respectively.

Table 1. Characteristics of raw rice mill effluent treated with immobilized *Pseudomonas* sp. cells in a packed bed reactor (0.9 mg cells / bead) with time

Parameters	Initial	17 hrs	20 hrs	24 hrs	40 hrs	44 hrs	48 hrs	65 hrs	68 hrs	72 hrs
pH	8.4	8.2	8.0	8.1	8.1	8.2	8.1	7.9	8.2	8.2
Temperature (°C)	29.7	30.3	31.1	32.6	31.0	31.0	31.8	31.0	31.1	31.4
Conductivity(mS)	11.4	2.37	2.46	2.50	2.44	2.41	2.46	2.44	2.43	2.44
TDS (ppt)	4.64	1.09	1.10	1.09	1.11	1.10	1.10	1.11	1.10	1.10
Salinity (ppt)	12.1	2.84	2.86	2.86	2.86	2.87	2.87	2.87	2.87	2.85
Turbidity (NTU)	431	276	281	323	344	340	342	328	326	319
COD (mg/l)	1400	464	440	624	656	640	840	896	688	856
DO (mg/l)	7.8	6.2	6.4	6.6	5.6	6.6	7.0	7.6	7.4	5.6
BOD (mg/l)	36	16	28	22	24	30	28	30	30	20

In reactor study 4 a cell loading of 2.2 mg per bead was used. The characteristics of the raw effluent after treatment by immobilized *Pseudomonas* sp. cells in a packed bed reactor for 24 hours are shown in Table 2. The electrical conductivity decreased by 82.044 %. The

salinity decreased by 82.01 %. Total dissolved solids decreased by 81.64 %. Turbidity decreased by 48.43 %. The percentage decrease in COD, DO, BOD was found to be 86.44 %, 22.45 % and 55.34 % respectively.

Table 2. Reduction (%) of the characteristics of raw rice mill effluent after 24 hour treatment with immobilized *Pseudomonas* sp. cells in a packed bed reactor

Parameters	Values (% reduction)		
	0.9 mg cells / bead	1.7 mg cells / bead	2.2 mg cells / bead
Electrical conductivity(mS)	78.72	81.80	82.04
Salinity (ppt)	76.53	80.81	82.01
Total dissolved solids(ppt)	76.55	81.02	81.64
Turbidity(NTU)	25.62	40.66	48.43
COD (mg/l)	55.51	70.40	86.44
DO (mg/l)	15.51	16.66	22.45
BOD (mg/l)	22.69	25.38	55.34

From the analysis of the results obtained with different cell loadings was found that the packed bed system with 2.2 mg cells per bead packing material improved the quality of the effluent standard. Further increasing of the cell loading does not improve the efficiency of the biodegradation. Because there is no significant difference between the results obtained from the reactor studies 3 and 4 we can conclude that the packed bed system with 2.0 mg cells per bead packing material is completely sufficient for optimal efficacy of treatment of rice mill effluent.

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## ÀÊÏ ÄÅÃÐÄÄÄÖÈË Í Á Î Ñ ÒÏ ÄÄÜ×Í È ÄÏ ÄÈ Ì ÐÈ Ì ÐÄÐÄÄÏ ÒÈÄÒÄ Í Ä Î ÐÈÇ Ñ ÈÌ Î ÄÈÈÈÇÈÐÄÍ È ÈÈÄÒÈÈ Í Ä PSEUDOMONAS SP.

Ð. Ì àí î ààðè, Ä. Äàí èàè, Ä. Èðóñòàí í à

Ðàçðè à: Çà ììéó-:ààí á íà yäèáí î ðèç í áí áóí àèì î òí é àà ñà ì ðäðäáí òè ñ òàè ì òñòäí yàáí á íà èðñíèòá è òðèòèòá. Õíçè ì ðí òáñ èçñèéàà áí èáí è èí èè-:áñòàà áí àà çà í àèèñááí á íà í áí áðäáí òáí èy î ðèç. Äèì òàçè áí àà í á áúáá ì ðäáèèí ì ðäðäáí òáí à òy ì ì æá àà ì ðäáèçàèèà ñáðèí çí è áèí èí àè-í è ì ðí áéáí è. Çà òàèòà ì èèðí áí àòà áèí ááðäááòèy ñá ì èàçàà í àé-í ì áóí äyüèy ì ì áóí ä. Í ò òàèèàà ì òí àáí è áí àè á èçí èèðáí è èááí òèòèòèðáí Ùàì Pseudomonas sp., èí èòí á èçí ì èçááí á í àñòí yüàòà ðááí òà ñ òàè áèí ááðäááòèy íà ì òí àáí è áí àè ì ðè ì ðäðäáí òèàòà íà ì ðèç. Ä ì ðäáááðèòèáèí è èçñèááááí èy ñà ì ðí ó-:áí è áèí ááðäááòèí ì ì èòá òàðàèòáðèñòèèè íà Ùàì à á ñáí áí ááí è èì í áèèèçèðáí áèä. Í ðè áèñí áðèì áí òèòá á áèí ðäáèòí ð ñ òèèñèðáí ñèí é áèí èàòàèèçàòí ð á ì ì ñòèáí àòà 86.44% ðäáòèòèy íà ÕÍ È è 55.34% ðäáòèòèy íà ÁÍ È. Ñúáúðæáí èáòí íà ðàçòáí ðèì è èí ì ì ì í áí òè í àì àèyàà çí à-èòáèí ì. Áèñí áðèì áí òèòá ñ ðàçèè-í à èí ì òáí òðàòèy íà èéáòèèòá á áèí èàòàèèçàòí ðà ì ì èàçààò, =á 1.7-2.2 ì á èéáòèè á ááí à -:áñòèòà èì ì áèèèçèðáí áèí èàòàèèçàòí ð ñà ì ì òèì áèí è çà áòáèòèáí ì ðä-:èñòááí á íà ì òí àáí è áí àè ì ðè ì ðäðäáí òèàòà íà ì ðèç