



DOI: <http://dx.doi.org/10.1590/1807-1929/agriambi.v24n5p343-347>

Rice husks as a microbial source for wastewater treatment

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ABSTRACT: Water contaminated with organic carbon and nitrogen compounds is one of the most important water problems in the contemporary world. The main problems related to the discharge of these compounds into natural environments are eutrophication and oxygen depletion. In view of this scenario, the objective of this work was to assess the use of rice husks for the production, growth, and propagation of microorganisms, and the use of these microorganisms for the removal of organic matter and nitrogen compounds in wastewater treatments. The following treatments were evaluated: industrial wastewater (IW), IW + rice husks, IW + sawdust and IW + dried leaves of trees. The effluent was treated with 3% (v v⁻¹) inoculum of microorganisms obtained from rice husks as substrate, applied in a batch system. A solution of 30 mg L⁻¹ of N-NO₃ containing 3% (v v⁻¹) of microorganisms obtained from rice husks was used as substrate to evaluate the reusability and performance of N-NO₃ removal. Higher microbial growth was found when the rice husks was used as substrate. The chemical oxygen demand, biochemical oxygen demand, total nitrogen, and total solid contents were reduced above 80%. The kinetic evaluation showed that total nitrogen and nitrate can be reduced by up to 80% after 72 h. Moreover, the reuse test showed the stability of the microbial activity after 7 days of use. This study showed that rice husks is an interesting and more sustainable source of carbon for microbial growth, with effective use in wastewater treatments.

Key words: pollutants, substrate, nitrogen removal, nitrate

Utilização da casca de arroz como fonte microbiana para tratamento de efluentes

RESUMO: A água contaminada com carbono orgânico e com compostos nitrogenados se apresenta como um dos mais importantes problemas hídricos do mundo contemporâneo. Os principais problemas relacionados à descarga desses compostos no ambiente natural são a eutrofização e a depleção de oxigênio. Diante desse cenário, o objetivo deste estudo foi utilizar a casca de arroz para geração, crescimento e propagação de microrganismos, e posteriormente, utilizar no tratamento de águas residuais para remoção de matéria orgânica e compostos de nitrogênio. Primeiramente, foram avaliados os seguintes tratamentos: água residual industrial (ARI), ARI + casca de arroz, ARI + serragem e ARI + folhas secas de árvores. Para o tratamento de um efluente real, 3% (v v⁻¹) de inóculo de microrganismos obtidos da casca de arroz como substrato foi aplicado em um sistema de batelada. Uma solução de 30 mg L⁻¹ de N-NO₃ contendo 3% (v v⁻¹) de microrganismos obtidos da casca de arroz como substrato foi utilizada para avaliar a reutilização e desempenho da remoção de N-NO₃. Verificou-se maior crescimento microbiano quando a casca de arroz foi utilizada como substrato. A demanda química de oxigênio, a demanda bioquímica de oxigênio, o nitrogênio total e os sólidos totais foram reduzidos acima de 80%. Os estudos cinéticos mostraram que o nitrogênio e o nitrato total podem ser reduzidos em até 80% após 72 h. Além disto, o teste de reutilização demonstrou a estabilidade da atividade microbiana após sete dias de utilização. Este estudo mostrou que a casca de arroz é uma fonte interessante e mais sustentável de carbono para o crescimento microbiano, com uso efetivo no tratamento de águas residuais.

Palavras-chave: poluentes, substrato, remoção de nitrogênio, nitrato



INTRODUCTION

Economic development and industrialization pose serious risks to drinking water availability on the planet (João et al., 2018). Contaminated water with organic carbon and nitrogen compounds is one of the most important water problems in contemporary world (Khan & Malik, 2019). The main problems related to the discharge of these compounds into natural environments are water eutrophication and oxygen depletion (Hulle et al., 2010).

Increasing water contamination by nitrogen-containing compounds requires special attention since there is a global concern about polluted water with ammonia, nitrite, and nitrate ions (Bartucca et al., 2016; Shen et al., 2016). This is probably the most widespread contaminant in groundwater because of the high solubility of nitrate in water, and is a serious risk to the adequate supply of drinking water and to human health (Costa et al., 2018). Organic and nitrogenous pollutants from wastewater are generally removed through biological treatments (Huang et al., 2017). This process involves transformation of dissolved and suspended organic contaminants into biomass and release of gases (Low & Chase, 1999).

Agricultural residues, such as rice husks, can be an abundant and cheap source of carbon and has been widely studied as a medium for the production, fixation and propagation of microorganisms (Akinyele & Adetuyi, 2005; Yang et al., 2015). However, most studies do not show the use of these microorganisms in wastewater treatments. Thus, the objective of this study was to assess the use of rice husks for the growth and propagation of microorganisms, and the use of these microorganisms for the removal of organic matter and nitrogen compounds in wastewater treatments.

MATERIAL AND METHODS

The study was carried out using rice husks collected at an agricultural cooperative of rice processing (COPAGRO), in southern state of Santa Catarina, Brazil. The microorganisms were immobilized in rice husks or suspended in solution. All chemical reagents used in analytical degree and solutions were prepared with deionized water obtained by the Millipore Milli-Q system.

The wastewater samples were collected monthly over one year at the COPAGRO wastewater treatment station. The wastewater was sampled according to the NBR 9897 - Planning of liquid effluent sampling and receiving bodies (ABNT, 1987a), and NBR 9898 - Preservation and sampling techniques of liquid effluents and receiving bodies (ABNT, 1987b).

The rice husk composition, regarding protein, extractives, ash, and moisture contents was determined in triplicate by laboratorial chemical analysis at the Universidade do Sul de Santa Catarina, Tubarão, SC, Brazil. Cellulose, hemicellulose, and lignin contents in the rice husks were determined using the van Soest method (Soest et al., 1991).

The microorganisms were obtained from the following biomass residues: industrial wastewater (IW), IW + rice husks, IW + sawdust, and IW + dried tree leaves. The experiment

was conducted by placing 200 mL of water in a sterilized erlenmeyer and 15% wb of each biomass was added. The tests were performed without additional source of carbon and nitrogen.

The inoculum prepared was kept in an oven for 72 h at 37 °C before the counting of colony forming units (colony forming units - CFU); 1 mL was taken every 24 h for dilutions of 1:10³ and 1:10⁶; 1 mL was seeded on Mueller-Hinton agar plates with a sterile Drigalski loop. The dilution was spread evenly across the plate and incubated in an oven for 24 h at 37 °C. The colonies were then manually counted on the plates. Eq. 1 was used to calculate the number of CFUs (CFU mL⁻¹):

$$\text{CFU} = \frac{\text{Number of colonies}}{\text{Dilution}} \quad (1)$$

The sowing on Mueller-Hinton agar allowed the observation of colony growth, which was separated from successive sowing on Macconkey and Blood agar plates to obtain pure cultures. The colonies were characterized morphologically (color, shape, elevation), subjected to GRAM staining and biochemical tests (catalase and oxidase). The bacteria were identified using identification kits (New Prov) in the UNISUL Biochemistry Laboratory (CENTEC), based on morphological and biochemical tests previously described by Silva et al. (2017).

The bacteria were then inoculated in brain heart infusion (BHI) to be later applied on bioreactors (batch) for evaluation in the industrial wastewater treatment using the parboiling process. The best results were obtained with the rice husk biomass.

Microorganisms were isolated from rice husk biomass as follows: rice husks were briefly washed under running water and incubated under anaerobic conditions at 37 °C in a 15% ratio (m v⁻¹). After incubation for 72 h, the solution was separated from the rice husks and centrifuged for 5 min at 6000 rpm. The supernatant containing water was discarded and the bacterial pellet was inoculated in a 250 mL flask containing distilled water. The bacterial pellet was then seeded on Blood Agar plates (0.5% of ovine blood) by exhaustion technique and incubated at 37 °C for 24, 48, and 72 h.

The control solution for microorganism propagation consisted of autoclaved rice husk biomass as the only source of carbon. The solution was incubated for 72 h.

Rice husk samples were washed under running water and mixed with nitrogen contaminated water (30 mg N-NO₃ L⁻¹) at 15% (m v⁻¹) in 250 mL erlenmeyer flasks. The solution was kept under a dubnoff orbital shaking for specific times at 25 °C. The control treatment consisted of sterilized biomass, autoclaved rice husks at 121 °C for 15 min before mixing in water contaminated with nitrate. The control treatment was performed in absence of external carbon source (rice husks). All assays were performed in triplicate.

The kinetic experiments to evaluate the nitrogen removal (N_{Total} and N-NO₃) were performed in the laboratory in a batch system. Synthetic wastewater was prepared using deionized water and ammonium acetate (P.A., Merck) or sodium nitrate (P.A., Merck). Total nitrogen removal was evaluated using an ammonium acetate solution with concentration of 40 mg L⁻¹

of nitrogen (N-NH₄). N-NO₃ removal was determined using a sodium nitrate solution with concentration of 30 mg L⁻¹ of N-NO₃. The experiment was conducted in 250 mL erlenmeyer flasks at 25 °C, pH 7 in a Dubnoff water bath, under constant stirring at 30 rpm. The aliquots were collected at intervals of 24, 48, and 72 h, which were adequate times for the proposed treatment. The total nitrogen and nitrate analyses were evaluated in triplicates using a Spectroquant Merck spectrophotometer (PHARO 300, Test kit).

The reusability and performance of the N-NO₃ removal by the microorganisms were evaluated using a new inoculum prepared and kept incubated at 37 °C for 72 h. Then, 100 mL of the 30 mg L⁻¹ of N-NO₃ solution with 3% (v v⁻¹) of the prepared inoculum was placed into erlenmeyer flasks at 25 °C, pH 7 in a Dubnoff bath, under constant stirring at 30 rpm. An aliquot was collected every 24 h and the system was fed with 30 mg L⁻¹ of N-NO₃ solution. This study lasted 7 days and all assays were performed in triplicate.

The conversion process was monitored through nitrate, total nitrogen, color, turbidity, sulfate, COD and solids analysis, as described in the Standard Methods for the Examination of Water and Wastewater (APHA, 2012) with analysis in spectrophotometer (spectrophotometer Spectroquant® Pharo 300 Merck) with a wavelength range of 190-1100 nm. The biochemical oxygen demand (BOD₅) was evaluated using *Oxitop* respirometers. The water pH was measured using a portable pH meter (Hanna). All assays were performed in triplicate.

RESULTS AND DISCUSSION

The main constituents of the rice husks were cellulose (34.61% weight basis - wb), lignin (19.66% wb), ash (16.82% wb), hemicellulose (10.90% wb), and protein (3.16% wb) (Table 1). The overall composition of the rice husks was similar to those of other studies (Chuah et al., 2005; Abbas & Ansumali, 2010; Lim et al., 2012). However, the comparison with these other biomasses showed that the rice husks evaluated in the present study had lower cellulose, lignin, and hemicellulose contents, and relatively more protein content.

The results of the microorganism production over time with the different biomasses are presented in Table 2. The industrial

Table 1. Chemical composition of the rice husk biomass

Constituents	% weight basis
Cellulose	34.61 ± 3.35
Lignin	19.66 ± 2.40
Ashes	16.82 ± 1.80
Hemicellulose	10.90 ± 1.55
Moisture	6.42 ± 1.10
Extractives	3.17 ± 0.68
Protein	3.16 ± 0.45

Table 3. Characterization of microorganisms produced in the rice husk biomass

Bacteria	Gram stain test	Oxidase	Catalase	Hemolysis in blood agar	Morphology
<i>Oerskovia</i> spp.	Positive Bacilli	Negative	Positive	Variable	Yellow colony
<i>Enterococcus</i> sp.	Positive Cocci	Negative	Negative	Negative	Punctiform colony
<i>Bacillus mycoide</i>	Positive Bacilli	Negative	Positive	Beta	Rhizoid colony
<i>Escherichia coli</i>	Negative Bacilli	Negative	Positive	Positive	Wet colony, white/transparent

Table 2. Evolution of microbial growth using different biomasses and industrial wastewater (IW)

Time (h)	IW	IW + rice husk*	IW + sawdust*	IW + dried leaves of trees*
0	-	-	-	-
24	1.12 × 10 ⁴	6.96 × 10 ⁷	2.22 × 10 ⁴	1.08 × 10 ⁵
48	1.64 × 10 ⁴	8.50 × 10 ⁷	2.32 × 10 ⁴	2.50 × 10 ⁵
72	2.71 × 10 ⁴	11.20 × 10 ⁷	3.96 × 10 ⁴	4.20 × 10 ⁵

CFU - Colony forming unity; *15% wb of the respective biomass was added; IW: Industrial wastewater

wastewater (IW) was used to evaluate microbial growth propagation. The wastewater without addition of biomasses also showed microbial growth. This was due to the presence of nutrients in the raw wastewater from the rice processing. The wastewater from rice parboiling can promote microbial growth, even without additional biomass.

Nevertheless, the addition of biomass (15% wb) enhanced microbial growth, especially when rice husks was used as substrate. The better performance of rice husks was probably due to its chemical composition (Table 1), which favored microbial growth. The lack of nitrogen source and excessive lignin content of the other tested biomasses may have hindered microbial growth, causing nutrient deficiency and formation of inhibitor by-products due to lignin degradation, respectively (Negi, 2012; Asina et al., 2017). Moreover, sawdust and dried leaves may release phenolic compounds when are in contact with water, which also inhibits microbial activity (Rauha et al., 2000; Cushnie & Lamb, 2011; Negi, 2012;).

The most important bacteria produced in the rice husk biomass were identified and characterized (Table 3), which were probably *Oerskovia* spp. (Gram positive Bacilli), *Enterococcus* sp. (Gram positive Cocci), *Bacillus mycoide* (Gram positive Bacilli), and *Escherichia coli* (Gram negative Bacilli). These bacteria have the unusual property of responding to variations by their mechanical strength on the surface structure of the growth medium with the formation of a biofilm. These biofilms, when organized transversely by a set of bacteria, may be responsible for the process of removal of contaminants (organic compounds and nitrogen) from wastewaters.

The untreated wastewater presented a high concentration of organic matter (Table 4), with averages of 4884 mg L⁻¹ for COD, and 1600 mg L⁻¹ for BOD₅. High values for color (220 Hz) and turbidity (176 FAU) were also observed. Color and turbidity are indicators of wastewater quality and are often interconnected. Thus, high contamination levels can be related to high color and turbidity values (Simões et al., 2008). The nitrogen (N_{Total}, 56.04 mg L⁻¹) and nitrate (N-NO₃, 7.4 mg L⁻¹) contents were significant. The nitrogen content may be mainly related to the presence of protein and acidogenic loads (nucleic acid) from the organic matter of vegetable origin. Similar results were

Table 4. Parameters analyzed for rice parboiling wastewater untreated and treated with 3% (volume basis - vb) of the inoculum (microorganisms) obtained from rice husks

Variables	Wastewater	Treated wastewater	Removal efficiency (%)
pH	5.5 ± 0.1	6.4 ± 0.2	-
Color (Hz)	220 ± 14	78 ± 4	64.50
Turbidity (FAU)	176 ± 8	64 ± 3	63.60
COD (mg L ⁻¹)	4,884 ± 132	1050 ± 53	78.50
BOD ₅ (mg L ⁻¹)	1,600 ± 58	480 ± 10	70.00
N-NO ₃ (mg L ⁻¹)	7.40 ± 1.2	3.4 ± 0.2	54.05
N _{Total} (mg L ⁻¹)	56.04 ± 8	9.5 ± 2	83.04
Sulfate (mg L ⁻¹)	6.70 ± 0.9	0.8 ± 0.3	88.05
Suspended solids (mg L ⁻¹)	302 ± 12	31 ± 4	89.70
Total solids (mg L ⁻¹)	23,426 ± 185	128 ± 10	99.45

BOD = Biochemical Oxygen Demand; COD = Chemical Oxygen Demand

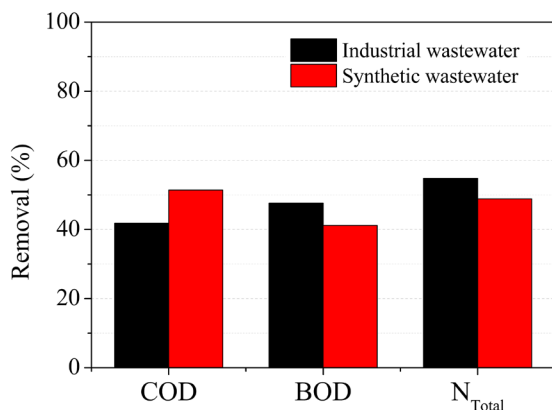
found in studies with wastewater from parboiled rice process employed as a medium for microalgae and cyanobacteria growth (Mukherjee et al., 2016).

All analyzed variables had, in general, average efficiency above 60% (Table 4) in the removal of contaminants. The greatest reductions were found for the removal of total solids (99.45%), suspended solids (89.70%), sulfate (88.05%), N_{Total} (83.04%), COD (78.50%), and BOD₅ (70%). The average color value was reduced from 220 to 78 Hz, and the turbidity from 176 to 64 FAU, representing an effective removal of approximately 64% of the organic matter from the degradation of the plant residue (rice husks).

Two experiments were conducted using different wastewaters and decreasing inoculum concentration up to 1% (v v⁻¹) to evaluate the efficiency of the inoculum.

The carbon and nitrogen organic matter decreased in both analyzed samples, industrial and synthetic wastewater (Figure 1). However, the samples that presented the best removals for the N_{Total} and BOD₅ were from the industrial wastewater, with reductions of 47.62 and 54.79%, respectively. However, the average removal of COD was 42%. These results were probably obtained because this type of wastewater already presented some nutrients in its composition.

An increase in microorganism concentration (active cells) in the medium was observed by the end of the experiment. The control treatment in absence of inoculum did not reduce the analyzed variables (N_{total}, COD, BOD₅).



BOD₅ - Biochemical Oxygen Demand; COD - Chemical Oxygen Demand

Figure 1. Removal of organic matter and total nitrogen from industrial and synthetic wastewaters after 72 hours, using 1% (vb) of inoculum of microorganisms

The period of greatest removal of N was up to 24 h of the experiment, in which N_{Total} was reduced from 40 to 25 mg L⁻¹, and N-NO₃ from 30 to 18 mg L⁻¹, representing removals of about 40% (Figure 2).

This was expected, since more nutrients are available to microorganisms at the beginning of the test, and a higher N concentration induces the microbial activity (Shuler & Kargi, 1992). Therefore, the rate of removal decreases progressively until reaching the equilibrium state (~80% of removal) after 72 h.

The evaluation of the reusability through continuous process of the microorganism inoculum is shown in Figure 3.

The removal of N-NO₃ during the 7 days of experiment showed that the microorganisms present a stable performance when fed with the same amount of substrate. The results were consistent to those previously discussed, with a removal around 80% of the initial N-NO₃ load. Therefore, the microorganisms produced from the rice husks have great potential to be employed on nitrogen removal from wastewaters.

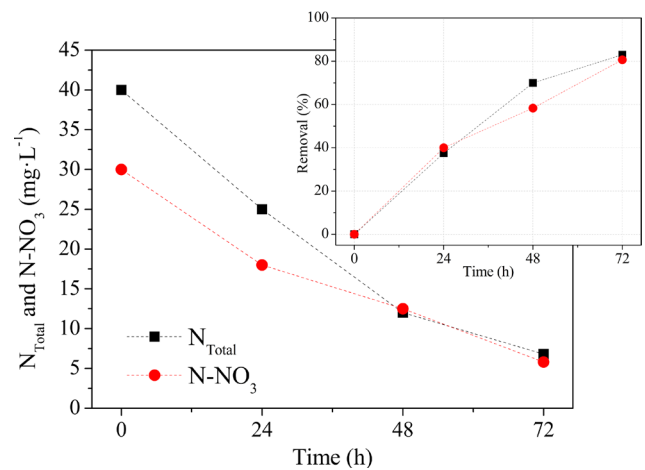


Figure 2. Concentration (mg L⁻¹) and removal (%) of total nitrogen and nitrate nitrogen over time, using 3% (vb) of inoculums of microorganisms

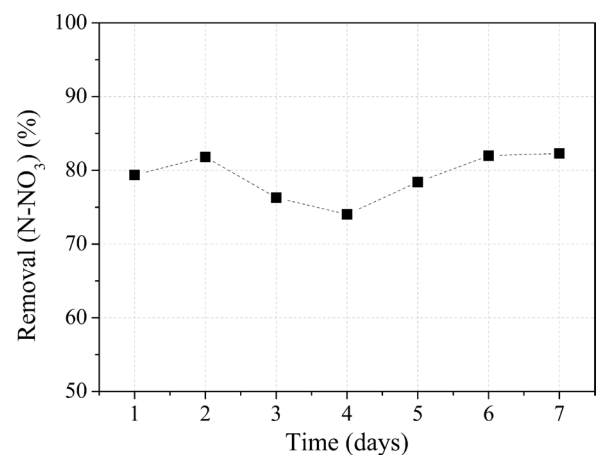


Figure 3. Continuous process test of the microorganism inoculum (3% vb) for the removal of N-NO₃ (batch concentration of 30 mg L⁻¹)

CONCLUSIONS

1. Rice husks is a promising biomass source for growing of microorganisms capable of treating wastewater.

2. The optimal amount and conditions for microorganism cultivation was 15% ($m v^{-1}$) of rice husks at 25 °C for 72 h. In general, the average efficiency of contamination removal exceeded 60%.

3. The variables total solid, N_{Total} , COD, and BOD_5 contents decreased in 99, 80, 78, and 70%, respectively, when using inoculations with 3% ($v v^{-1}$) of microorganisms obtained from rice husks as substrate.

4. The inoculum presented a stable and continuous activity even after 7 days of the batch experiment, denoting the potential and reproducibility of nitrate removal.

ACKNOWLEDGMENTS

The authors thank the Universidade do Sul de Santa Catarina, Fundação de Amparo à Pesquisa e Inovação do Estado de Santa Catarina, and Conselho Nacional de Desenvolvimento Científico e Tecnológico for the financial support to this project.

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