



# Parboiled rice effluent: A wastewater niche for microalgae and cyanobacteria with growth coupled to comprehensive remediation and phosphorus biofertilization

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## ARTICLE INFO

### Article history:

Received 9 April 2016

Received in revised form 3 June 2016

Accepted 7 September 2016

### Keywords:

Microalgae and cyanobacteria

Parboiled rice mill effluent

Bioremediation

Phosphorus biofertilizers

Consortium

Polyphosphate

## ABSTRACT

The potential of microalgae and cyanobacteria for bioremediation of wastewater by nutrient uptake combined with simultaneous biomass production is a well recognized perception of today's world. The present study illustrates the treatment of a highly polluted wastewater generated during parboiling of paddy in rice mill industries, widely operational in developing countries where rice is the staple food crop, with the help of microalgal and cyanobacterial isolates capable of growing at high rates in parboiled rice mill effluent (RME). This endeavor leads to comprehensive bioremediation of the said effluent and subsequent use of the harvested biomass as slow release phosphorus biofertilizers and the treated effluent for crop irrigation. The RME-acclimatized algal consortium demonstrated highest growth in terms of fresh weight and greatest remediation efficiency at the end of 36 days' treatment of RME, with 93.9% phosphorus and 100% ammonia-nitrogen removal, 98.7%, 91.6% and 93.5% reduction in biological oxygen demand, chemical oxygen demand and total dissolved solid, respectively, and an increment of  $186 \pm 0.3 \text{ mg L}^{-1}$  dissolved oxygen, bringing down the pollutants level well below the discharge limits suggested by Central Pollution Control Board, India. Additionally, microalgae in the consortium aggregated in clumps spontaneously in presence of the filaments of *Phormidium* sp. facilitating easy harvest. The RME-acclimatized algal consortium demonstrated highest accumulation of polyphosphate (poly-P) ( $0.76 \pm 0.01\%$  of dry weight) as well as highest release of phosphorus in non-sterile soil emphasizing the essential role of soil phosphorus solubilizing organisms to leach soluble phosphorus from the insoluble poly-P present in the biomass. The rice seedlings watered with treated RME also showed improved growth effect on shoot height and leaf width. The study results establish the suitability of RME as an excellent growth media for microalgae and cyanobacteria.

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## 1. Introduction

The magnificent potential of microalgae and cyanobacteria to treat wastewaters through nutrient removal and subsequent biomass production has been explored and reported by many researchers [1–6]. Most of the wastewaters, municipal or industrial are characteristically rich in carbon, nitrogen (N) and phosphorus (P), the key elements needed for growth of microalgae and cyanobacteria. Carbon, in the form of carbon dioxide is readily utilized by microalgae in autotrophic mode while in heterotrophic mode the organisms consume the organic form of carbon by reducing the chemical oxygen demand (COD) in wastewaters [5]. In certain microalgae like *Chlorella* sp., heterotrophic growth proceeds faster [7,8] decreasing the wastewater COD very swiftly.

Inorganic and organic nitrogen of the effluents are assimilated simultaneously by algae although ammonia is the preferred form of nitrogen [5] with nitrate being utilized after ammonia depletion [9]. Phosphorus, primarily in the form of phosphates, is another integral nutrient component of wastewaters utilized by microalgae. The concentrations of inorganic phosphates are usually low except for agricultural effluents which have high phosphates due to the presence of fertilizers [10]. It is suggested that nitrogen-to-phosphorus (N/P) ratio rather than the absolute concentration of nitrogen and phosphorus controls the growth of microalgae and cyanobacteria in the effluents [5]. The range of N/P ratios is typically 4.0–5.0 for most wastewaters but the favorable range of N/P ratio required for algal growth varies from species to species [5]. *Chlorella vulgaris*, one of the most widely grown microalgae in wastewater, shows excellent flexibility to exploit a wide range of N/P ratios to acclimatize easily in both autotrophic and heterotrophic mode. Although an optimal N/P ratio of 7.0 has been reported for *C. vulgaris*

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[11], N/P ratios of 2.0 and 8.0 [4] as well as N/P ratio as low as 0.36 [2] in municipal wastewater streams are also reported to be appropriate for its cultivation. In contrast, cyanobacteria are reported to favor low N/P ratios for maximum nutrient removal and optimum growth [5]. The phosphate removal rate of *Phormidium bohneri* grown in secondary municipal effluent increased 8.6-fold when the N/P ratio decreased from 6 to 1 [12]. Similarly, the highest removal of N and COD took place at a N/P ratio of 1.98 for *Aphanothece microscopica Nageli* [13]. Parboiled rice mill effluent (RME) is typical phosphorus rich wastewater developed in the parboiling process of paddy [14], where the N/P ratio varies from 2.0–5.0 with ammonia-nitrogen ( $\text{NH}_3\text{-N}$ ) contributing as the major nitrogen source and possesses high potential to act as a growth medium for microalgal and cyanobacterial cultivation.

Parboiling of rice is a hydrothermal process where the starch present in crystalline form swells, gets gelatinized and is transformed into an amorphous form [15]. The practice of parboiling involves soaking of paddy in large quantity of fresh water, steaming and drying prior to milling [16]. This traditional treatment process is practiced in many parts of the developing world like in India, Bangladesh, Pakistan, Myanmar, Malaysia, Sri Lanka, Brazil, Ghana, Nigeria, Guinea, South Africa and Thailand [17], where rice is the staple food crop. In India, Bangladesh, Pakistan and Sri Lanka, this wastewater is reported to be discarded without any remediation treatment into open crop fields or close by water bodies causing significant eutrophication, surface and ground water pollution, and wastage of large quantities of utilizable water [18–20].

Ground water levels in various parts of India are declining as the country is unable to adequately recharge aquifers in deficit areas. India has, at present, annual potential of  $112.3 \times 10^{13}$  L of 'utilizable' water with  $69 \times 10^{13}$  L coming from surface water resources and remaining  $43.3 \times 10^{13}$  L from ground water resources [21]. The Central Ground Water Board of India has reported that around 56% of the wells, which are analyzed to keep a tab on ground water level, showed decline in their level in 2013 as compared to the average of preceding 10 years (2003–12) period [21]. India will not be able to meet the future water demand of its population unless it recharges its water bodies adequately and uses water judiciously adding a thoughtful concern towards recycling of wastewater.

In view of the above, wastewater generated in parboiled rice mills of India is really huge; where the total volume of RME amounts to  $200 \times 10^5$  L per rice mill annually assuming lowest possible average outflow of effluent from each parboiling rice mill at  $1 \times 10^5$  L/day for 200 days/year [22]. In the state of West Bengal in India, having 16,925 functional rice mills,  $3,385,000 \times 10^5$  L water is wasted as RME per year [14]. In Sri Lanka, approximately  $6.04 \times 10^5$  L of effluent per 8 MT (million tons) of soaked paddy [23] and in Brazil,  $0.02 \times 10^5$  L of RME per MT of rice [24], is discharged into the environment. This RME is reported to be highly polluted in nature, harboring a wide range of pollutants like high COD  $1350\text{--}7809$   $\text{mg L}^{-1}$ , biological oxygen demand (BOD)  $510\text{--}4580$   $\text{mg L}^{-1}$ , total suspended solids (TSS)  $184\text{--}5134$   $\text{mg L}^{-1}$ , total dissolved solids (TDS)  $1386\text{--}3360$   $\text{mg L}^{-1}$ , color  $200\text{--}950$  chloroplatinate color units [25–29] with  $30\text{--}360$   $\text{mg L}^{-1}$  P concentration [28,30–31] and  $37\text{--}154$   $\text{mg L}^{-1}$   $\text{NH}_3\text{-N}$  concentration [28,31], has all the characteristics necessary for algal and cyanobacterial growth. Interestingly, this effluent does not contain any toxic metal contaminants [31,32] and this feature attracted scientists to explore the suitability of this wastewater as algal and cyanobacterial growth medium.

Significant attempts have been carried out worldwide to exploit this large amount of waste effluent, as a growth medium coupled to its remediation. Research groups from Brazil [13,33–34] used cyanobacterium *A. microscopica Nageli* for removal of nitrogen, COD and organic matter from parboiled wastewater and combine single-cell protein production with treatment of the effluent. Studies from India [35,36] explored the potential of RME as a growth medium for *Spirulina platensis*, *Scenedesmus abundans* and *Chlorella pyrenoidosa* [31] with

simultaneous P and ammoniacal nitrogen ( $\text{NH}_4\text{-N}$ ) removal and reduction of BOD during the course of growth. A study from Brazil [24] used *Pichia pastoris* X-33, a methylotrophic yeast as a bioremediator of parboiled RME.

The enormous volume of polluted water released regularly into the surroundings from parboiled rice mills not only poses a threat to the environment but this regular massive loss of fresh water/ground water is alarming and unacceptable in the backdrop of declining ground water levels in various parts of the world. Several treatment options like effluent treatment plants (ETPs), physico-chemical treatment technologies, biological remediation strategies, etc. are being developed from time to time, although they are yet to become mill owners-friendly in terms of establishment cost and running expenditure [14,37].

With this background scenario, the present study is an attempt to exploit the remediation power of the microalgae and cyanobacteria growing in parboiled rice mill wastewater coupled to the harvest of biomass at the end point of remediation and their use as slow release P biofertilizer showing promising results as compared to conventional chemical fertilizers. This phenomenon of algal remediation combined with the course of algal growth can turn out to be a cost effective alternative to expensive ETPs in parboiled rice mills of developing countries [14]. Additionally, in this study, the suitability of RME as an algal growth niche is emphasized and reuse of the treated wastewater for watering crops without causing any harmful effects is also advocated as it adheres to the limits for pollutants defined by the Central Pollution Control Board (CPCB), India. Our group is engaged in this research since a long time [14], using microalgae like *Chlorella* and *Parachlorella*, and cyanobacteria like *Cyanobacterium*, *Lyngbya* and *Phormidium*, for a comprehensive bioremediation of the effluent through the simple technique of eutrophication.

## 2. Materials and methods

### 2.1. Parboiled rice mill effluent collection

Effluent samples from the parboiled rice mills were collected in sterilized containers. Sampling was carried out in 113 rice mills spread over three districts of West Bengal, India (Table S1) namely, Hooghly (RME collected from 33 rice mills, Fig. S1), Burdwan (RME collected from 50 rice mills, Fig. S2), and Birbhum (RME collected from 30 rice mills, Fig. S3). The RME samples were transported to laboratory in ice-cold conditions within 2 h and stored at  $4^\circ\text{C}$ , till further analyses. Out of the collected RMEs, one particular RME was used for the growth experiments with microalgae and cyanobacteria due to its regular availability and adequate P ( $47.32 \pm 2.56$   $\text{mg L}^{-1}$ ) and  $\text{NH}_3\text{-N}$  ( $141.66 \pm 2.96$   $\text{mg L}^{-1}$ ) content with COD ( $1666 \pm 56.57$   $\text{mg L}^{-1}$ ) expected to support growth of the test organisms. Moreover, this parboiled rice mill happened to be our industrial collaborator in this research.

### 2.2. Characterization of RME

Different parameters of all the RME samples were measured and recorded. pH and electrical conductivity (EC) of the samples were measured using  $\mu\text{C}$  pH System 361 (Systronics, Ahmedabad, India) and Digital Conductivity Meter CI 250 (Chemiline, Ahmedabad, India), respectively. Phosphorus concentration of the RME samples was determined by Molybdenum Blue method [38]. Ammonia-nitrogen concentration of the RME samples was determined by Phenate method, measuring the indophenol blue color [39]. Nitrate-nitrogen concentration was determined by measuring the absorbance of nitrosalicylic acid [40]. DO was measured by Winkler method with azide modification, BOD was measured by titrimetric method, COD was measured by open reflux method, and TDS was measured by high temperature evaporation and desiccation method, following the guidelines of Central Pollution Control Board (CPCB), India [41]. Reducing sugar content was measured spectrophotometrically using anthrone reagent [42].

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