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

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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 ± 0.3 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 ± 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.

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], [2], [3], [4], [5], [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* [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 to1 [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 (NH_3-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], [19], [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 112.3×10^{13} L of ‘utilizable’ water with 69×10^{13} L coming from surface water resources and remaining 43.3×10^{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 200×10^5 L per rice mill annually assuming lowest possible average outflow of effluent from each parboiling rice mill at 1×10^5 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 6.04×10^5 L of effluent per 8 MT (million tons) of soaked paddy [23] and in Brazil, 0.02×10^5 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 1350–7809 mg L^{-1}, biological oxygen demand (BOD) 510–4580 mg L^{-1}, total suspended solids (TSS) 184–5134 mg L^{-1}, total dissolved solids (TDS) 1386–3360 mg L^{-1}, color 200–950 chloroplatinate color units [25], [26], [27], [28], [29] with 30–360 mg L^{-1} P concentration [28], [30], [31] and 37–154 mg L^{-1} NH_3-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 (NH_4-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.

Section snippets

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 °C, till further analyses. Out of the

Characterization of RME

The different physico-chemical parameters of the 113 RME samples are recorded in Table 1. The effluents were dark yellow to brown in color with highly acidic pH, having high TDS, BOD and COD resulting in low DO. The effluent is devoid of any toxic metals as reported in the earlier studies [31], [32] but as evident from the observed parameters, have high P and NH_3-N concentration. Therefore, this polluted effluent was expected to be suitable for microalgae and cyanobacteria culture experiments.

Identification of the microalgal and cyanobacterial isolates

Conclusion

The entire study advocates the suitability of parboiled rice effluent as a competent growth medium for microalgae *Chlorella* sp. (KJ654316, KJ654314) and *Parachlorella* sp. (KU990883) and cyanobacteria *Lyngbya* sp. (KF644563), *Phormidium* sp. (KU740239) and *Cyanobacterium* sp. (JX023443). Moreover, all the six organisms as a consortium not only showed the best productivity in terms of fresh weight, their growth finally ended up with the best comprehensive bioremediation, greatest P accumulation and

Conflict of interest

The authors declare no conflict of interest.

Author contribution

CM conceptualized and designed the work, isolated strains, performed the experiments, gathered data, interpreted and analyzed the results and finally drafted the manuscript. RC isolated strains, performed experiments, analyzed and interpreted data. TS, MB and SMG each have significant contribution in isolation of strains, collection of RME samples and designing of the experiments. SKB has provided logistic and scientific support in collection of RME samples and designing of the work. KR as a

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- **A comparative study on the inhibitory effects imposed on earthworms by brewery and rice mill wastewater**

  2023, Journal of Environmental Chemical Engineering

  Show abstract

  Of late, vermifiltration (VF) technology has gained major attention from researchers as a sustainable wastewater treatment alternative in which the treatment of wastewater has been driven by earthworms (EWs). Apart from parametric effects, the composition, biodegradability/complexity, hydraulic and organic loading rates (HLR/OLR), and the concentration of organics present in wastewater exhibit inhibitory effects on EWs, declining the treatment efficiency of vermifilters (VFs), which have not been adequately explored. Hence, the present study dealt with the assessment of such inhibitory effects on the survival, growth, and reproduction of EWs using four kinds of wastewater. Results indicated that EWs sustained the flow of rice mill, real brewery, synthetic brewery, and the mixture of real brewery and domestic wastewater for 5–11, 11–21, 20–40, and 23–45 d, respectively, before reaching 50% mortality, at the HLR of 8–4 m^3/m^2-d. For each wastewater, the lowest HLR (4 m^3/m^2-d) yielded the highest growth of EWs. The mixture of real brewery and domestic wastewater provided the most favorable conditions for the reproduction of EWs, ensuring the highest number of cocoons (8–12 nos./EW) and juveniles (11–26 nos./EW) produced per EW, followed by synthetic brewery (cocoon: 3–9 nos./EW; juvenile: 5–18 nos./EW), real brewery (cocoon: 2–7 nos./EW; juvenile: 2–12 nos./EW), and rice mill wastewater (cocoon: 1–5 nos./EW; juvenile: 2–8 nos./EW), with the HLR varying inversely with the reproduction rate of EWs. Hence, the mixture of real brewery and domestic wastewater portrayed the least biological inhibition on EWs, whereas rice mill wastewater had the highest. Outcomes of the present study revealed that the VF technology is best suited for remediating the mixture of real brewery and domestic sewage, can substantially remediate synthetic brewery wastewater, and may treat real brewery and rice mill wastewater after an appropriate pretreatment. The premixing of real brewery wastewater with rapidly biodegradable and low-strength domestic sewage at a specific volumetric ratio improved the treatability of the former using the VF technology attributed to the enhanced biodegradability and reduced organic strength of the wastewater mix.

- **Bioremediation strategies of palm oil mill effluent and landfill leachate using microalgae cultivation: An approach contributing towards environmental sustainability**

  2023, Chinese Chemical Letters

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  Palm oil mill effluent (POME) is defined as the wastewater that contains high concentrations of organics, nutrients and oil and grease generated from the production process of palm oil. Therefore, proper discharge and management of POME is important to avoid deleterious impact on the environment. In fact, solid waste generation is a precursor for its disposal issues as most of the solid waste generated in developing nations is dumped into landfills. This has led to the threat posed by the generation of landfill leachate (LL). LL is a complex dark coloured liquid consisting of organic matter, inorganic substances, trace elements and xenobiotics. Hence, it is essential to effectively treat the landfill leachate before discharging it to avoid contamination of soil, surface & groundwater bodies. Conventional treatment methods comprises of physical, biological and chemical treatment, however, microalgal-based treatment could also be incorporated. Furthermore, with the benefits offered by microalgae in valorisation, the application of microalgae in POME and leachate treatment as well as biofuel production, is considerably viable. This paper provides an acumen of the microalgae-based treatment of POME and LL, integrated with biofuel production in a systematic and critical manner. The pollutants assimilation from wastewater and CO_2 biosequestration are discussed for environmental protection. Cultivation systems for wastewater treatment with simultaneous biomass production and its valorisation, are summarised. The study aims to provide insight to industrial stakeholders on economically viable and environmentally sustainable treatment of wastewaters using microalgae, and eventually contributing to the circular bioeconomy and environmental sustainability.

- **Roles of microalgae-based biofertilizer in sustainability of green agriculture and food-water-energy security nexus**

  2023, Science of the Total Environment

Show abstract

For years, agrochemical fertilizers have been used in agriculture for crop production. However, intensive utilization of chemical fertilizers is not an ecological and environmental choice since they are destroying soil health and causing an emerging threat to agricultural production on a global scale. Under the circumstances of the increasing utilization of chemical fertilizers, cultivating microalgae to produce biofertilizers would be a wise solution since desired environmental targets will be obtained including (1) replacing chemical fertilizer while improving crop yields and soil health; (2) reducing the harvest of non-renewable elements from limited natural resources for chemical fertilizers production, and (3) mitigating negative influences of climate change through CO_2 capture through microalgae cultivation. Recent improvements in microalgae-derived-biofertilizer-applied agriculture will be summarized in this review article. At last, the recent challenges of applying biofertilizers will be discussed as well as the perspective regarding the concept of circular bio-economy and sustainable development goals (SDGs).

- **Recycling of wet grinding industry effluent using effective Microorganisms™ (EM)**

  2023, Heliyon

  Show abstract

A considerable volume of effluent released from the food processing industries, after the extensive use in the products manufacturing and industrial process. Effluents, either without treatment or with improper treatment, released out from the industries would severely damage the environment and human health. An investigation was done by recycling the effluent samples, collected from the wet grinding industry, Madurai, India, which was determined with an acidic pH (5.93), high turbidity (160.78 NTU), high BOD (62.4 mg/l) and COD (274.38 mg/l) and a significantly higher quantity of starch (115.81 mg/l). Biological wastewater treatment method was chosen in this experiment on the basis of the biodegradability index of effluent (3.21–10.75). The main goal of this study was to evaluate the effectiveness of wastewater treatment in a prototype STP utilizing the Effective Micro-organisms™ Consortium application. The US EPA International Water Quality Standards and the Water Quality Index were used to compare the water quality of the recycled effluent with and without the EM application. The effluent from the EM consortium treatment was found to have acceptable levels of pH (7.38), salinity (1.94 ppt) and Conductivity (4.05 mS); and a declining trend found in TDS (1.81 ppt), BOD (24.4 mg/l) and COD (148.83 mg/l) level when the effluent treated using EM. Removal effectiveness of EM significant reduce in the treated effluents starch (85.15%), sulphate (78.42), phosphate (79.60), nitrogen (65.54%), and turbidity (82.73%) level were observed. Which was shown to be comparatively better than employing without EM treatment. This research substantially intends to the best practices, towards sustainable industries through Cleaner Production Mechanism.

- **[Closed loop bioeconomy opportunities through the integration of microalgae cultivation with anaerobic digestion: A critical review]**

  2023, Bioresource Technology Reports

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  Bioenergy and bioproduct production can support energy-environment nexus that leads to a sustainable closed-loop economy. Particularly, manure management can be a very demanding, expensive and potentially limiting issue for livestock/poultry operations, especially in cold-regions. Clear steps must be taken towards sustainable nutrient management, reuse and recycling to add a source of income for farmers and improve environmental agricultural sustainability. Integration of clean-technologies, such as anaerobic digestion and microalgae cultivation, is essential to concentrate and recycle nutrients from fertilizers, reuse all manure fractions, and capture new value. This review aspires to (i) provide comprehensive information on integration potential of microalgae into a growing biogas industry, (ii) identify opportunities and challenges, (iii) review potential bioproducts prospect, and (iv) examine life-cycle and techno-economic assessments suitable for cold regions. Such integration can harness the huge untapped potential of the biogas-industry in a way that embraces the region-based circular economy and supports sustainable development goals.

- **[Co-cultivation of microalgae-cyanobacterium under various nitrogen and phosphorus regimes to concurrently improve biomass, lipid accumulation and easy harvesting]**

  2022, Biochemical Engineering Journal

  Citation Excerpt :

  Based on the superior biomass and lipid content, the optimal condition was subsequently utilized for the co-cultivation experiment. To identify the microalga and cyanobacterium strains, genomic DNA was extracted using the CTAB method prescribed by Mukherjee et al. [26]. Isolated DNA samples were stored at − 20 °C.