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**ANALYSES OF PHYSICO-CHEMICAL CHARACTERISTICS OF RICE MILL
EFFLUENTS AND ITS UTILIZATION IN ALGAL BIOMASS PRODUCTION**

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ABSTRACT

Rice mill is one of the most important agro based industries in India and is highly responsible for creating significant impact on rural economy in particular and countries economy in general. Rice mill is playing an important role in the economic development of the Indian sub-continent, but the effluents released produce a high degree of organic pollution in both aquatic and terrestrial ecosystems. They also alter the physico-chemical characteristics of the receiving aquatic bodies and affect aquatic flora and fauna. In the present study, the physico-chemical characteristics of ADT-43 and BPT rice processing effluent were analyzed. The soaked rice mill effluent (RME) revealed an alkaline pH (7.5), salinity (2 ppt), DO (12 mg L⁻¹), nitrate (0 µmol/l), nitrite (32.5 µmol/l), ammonia (93.6 µmol/l), phosphate (58.6 µmol/l), silicate (28.4 µmol/l), calcium (20 mg L⁻¹) and magnesium (1.1 mg L⁻¹). The boiled rice mill effluent revealed an alkaline pH (7.06), salinity (2 ppt), DO (9.15 mg L⁻¹), nitrate (17.98 µmol/l), nitrite (2.7 µmol/l) ammonia (9.18 µmol/l), phosphate (2.38 µmol/l), silicate (4.31 µmol/l), calcium (12 mg L⁻¹) and magnesium (2.24 mg L⁻¹). The physico- chemical characteristics of ADT-43 and BPT soaked and boiled rice effluent was compared each other and also selection of promising

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microalgae species based on their growth for bioremediation studies and that effluent will be used as an alternative medium for the culture of microalgae.

Keywords: Rice mill effluents, Physico-chemical properties, Microalgae, Nutrients, Bioremediation

INTRODUCTION

Environmental pollution has been recognized as one of the major problems of the modern world. Water is indispensable and one of the precious natural resources of our nation. The modern civilizations, over exploitations, rapid industrialization and increased population have led to fast degradation of our water resource [1]. Wastewater released from any industry has impact on water and land. It is very much necessary to increasing awareness of the fact that clean environment is necessary for smooth living and better health of human beings. Rice is one of the most important crops of the world. More than half of the world's population is supported by rice [2]. Among agro- industries, rice processing industry is the biggest industry in India. Almost all of the rice mill effluent generation comes from parboiling of rice. Approximately 1-1.2 L of effluent is discharged from per kg of paddy. Waste water coming from rice mill operations contains high concentration of organic and inorganic substance causing significant polluting phenomena [3]. Treatment of this effluent is a serious environmental issue.

Disposal on land is a common practice which results in nutrient overload in soil [4]. They also alter the physico-chemical characteristics of the receiving aquatic bodies and affect aquatic flora and fauna. These contain high amounts of nitrogen and phosphorus; so the effluents are not a suitable nutrient source for terrestrial crops as they can be easily leached from soil and cause eutrophication of surface water. The major chemical pollutants in rice mill effluent are nitrogen, phosphorus, heavy metals, detergents, pesticides, and hydrocarbons. The presence of nitrogen in rice mill effluent discharged can be undesirable because it has ecological impact and also affect public health. The microalgal cells have the capable to take nutrients such as phosphorus, nitrogen, and ammonium as well as heavy metals to reduce BOD in effluent. Microalgae play a vital role in the definition of one planetary boundary, namely the "biochemical flows". The flow of nutrients into water bodies stimulates algae growth [5]. In the present study, the physico-chemical characteristics of ADT-43 and BPT rice

waste water were investigated and focus on isolation and identification of waste water grown microalgae and selection of promising microalgae species for bioremediation studies.

MATERIALS AND METHODS

Area of the study and effluent collection:

The ADT 43 and BPT soaked and boiled rice mill waste water sample were collected using the sterilized containers from the tank of a rice mill industry located at Vilar, Thanjavur District, Tamil Nadu. The waste water was screened through coarse and fine screens to remove floating solids and processed in the lab under controlled condition with the help of ice box and the effluents were stored at 4°C to avoid changes in its characteristics.

Analysis of physico-chemical characteristics of ADT-43 and BPT RME samples:

The physico-chemical characteristics of the ADT and BPT soaked and boiled RME sample were analyzed using standard method of APHA [6] for pH, Dissolved oxygen, calcium, magnesium and total alkalinity. Nitrate was estimated using the method of Jenkins and Medsker [7]. Strickland and Parsons [8] method was followed for determining ammonia, phosphate, nitrite, silicate.

Preparation and maintenance of inoculum

Under aseptic conditions and using sterile metallic pins, individual colonies were picked and streaked into a new Conway's agar plates. The microalgae was cultured in special air conditioning room. The stock cultures were kept in 1 and 2 litres culture flasks. The tap water was filtered using filter bag (1 micron), the filtered water was sterilized by using autoclave and after cooling, water was transferred to the culture flask. Culture flasks are plugged with cotton or covered by aluminum foil. All vessels used for algal culture was sterilized properly and dried in an oven before use. Conway's medium was used for indoor culture.

Screening and selection of promising microalgae:

The six fresh water microalgae strains such as *Chlorella vulgaris* (CV), *Cosmarium* sp, (COS) *Scenedesmus product* (SPr), *Scenedesmus peccensis* (SPe), *Chlorella* sp.,(CHL) *Chloromonas* sp.,(CHM) were used in this study. A 10 ml of inoculum in the growing phase was transferred to the culture flasks with waste water and culture was provided with 12:12 hrs light and dark cycle with 5000 lux using two tube lights. The temperature was maintained in the range of 23 to 25°C for entire culture period. The

culture was provided with continuous aeration.

RESULT AND DISCUSSION

Characteristics of untreated rice mill effluent:

The physico- chemical characteristics of the ADT 43 and BPT soaked and boiled RME has been given in **Table 1 and 2**. The appearance of ADT 43 and BPT soaked and boiled RME was found to be brown and turbid. The turbidity is due to the shape, size and refractive index of the particulate impurities like finely 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. The smell or odour of the both RME was found to be unpleasant or foul. The offensive odour may be due to the volatile substances associated with organic matter and anaerobic decomposition by living organisms, principally microorganisms. The offensive odour impairs the water quality and causes nausea and vomiting.

Temperature is the measure of hotness of any material. The measurement of temperature in water is important basically for its effects on the biochemical reactions in

the living organisms. It is also important in the determination of pH, conductivity and saturation level of gases in water. In the present study, temperature of ADT 43 Soaked and Boiled rice mill water was 25°C and 27°C respectively; BPT soaked and boiled RME was 28°C and 25°C respectively. pH is the measurement of hydrogen ion concentration and it indicates instantaneously the intensity of acidity or alkalinity in water or effluent. It effects many chemical reactions and biological systems function only in relatively narrow pH ranges. The hydrogen ion concentration of water are influenced not only by the dissociation of water, but also by the relationship between the concentration of carbonic acid (H_2CO_3) and its ions (H^+ , CO_3^{2-}) as well as by the generation of these ions from the humic acids and by the hydrolysis of heavy metal salts. From our observation it was found that the effluent of rice mill is highly alkaline in nature and the pH varies from 6.5 to 8.8; 7.05 to 7.06 in ADT 43 and BPT soaked and boiled RME. The alkaline pH of the effluent may be due to reaction of area and common salt with phenols released from rice during boiling operations. The area used to bring glaze of rice in boiler tank broken down to CO_2 and NH_3 . The NH_3 in turn reacts with water and phenol to form ammonium,

phenolate, which are basic in nature. Besides these reactions, the sodium ions of NaCl (common salt is used in the boiler tank to suppress the boiling point of water) also reacts with phenol to form an alkaline substance called sodium phenolate which again increases the pH [9].

Alkalinity of the water is its capacity to neutralize strong acid and is characterized by the presence of all hydroxyl ions capable of combining with the hydrogen ion. Alkalinity in natural waters is due to free hydroxyl ions and hydrolysis of salts formed by weak acid and strong bases, such as carbonate and bicarbonates. The total alkalinity of ADT 43; BPT soaked and boiled rice mill waste water was 27 and 4 mg/L; 3 and 2mg/L respectively. Phosphorus occurs in regular and effluent almost as phosphate. The most significant sources of phosphate are the release of domestic sewage, industrial waste and agricultural run-off. Phosphorus is vital to the growth of organism and can be the nutrients that limit the primary efficiency of water pollution. The phosphate concentration of the ADT43 soaked and boiled RME varied between 10.9 and 0.36 $\mu\text{mol/l}$; BPT soaked and boiled rice effluent showed 58.6 and 2.38 $\mu\text{mol/l}$ respectively. Compared with ADT 43 sample BPT showed the higher phosphate concentrations.

Nitrate signifies the highest oxidized form of nitrogen and sources of nitrate is biological oxidation of organic substances, which come in sewage and industrial waste. Higher amount of nitrate is generally the indicator of pollution and excess of nitrate in water causes diseases in animals and methamoglobinemia in blood [10]. In the present study the nitrate content of rice mill waste water varied between 1.75 and 8.66 $\mu\text{mol/l}$; 0 and 17.98 $\mu\text{mol/l}$ in ADT 43; BPT soaked and boiled RME samples respectively. Ammonia, silicate and nitrite values were 12.69-6.54 $\mu\text{mol/l}$, 13.69-16.3 $\mu\text{mol/l}$, 29.19-0.38 $\mu\text{mol/l}$ in ADT 43 soaked and boiled RME samples respectively. In our study, physic-chemical parameters and nutrients were higher in the soaked RME sample and therefore this sample was chosen for screening and optimization studies. Among the soaked and boiled RME samples, the soaked RME samples was highly used in bioremediation studies and also used as an alternative medium for the culture of microalgae as agreed by earlier worker [11].

Selection of promising microalgae based on their growth in the waste water

Nitrogen and phosphorus are essential components for the growth of algae and are commonly present in effluent [12]. This study suggests that algae can be beneficial in

removing the excess nitrogen and phosphorus present in effluent through absorption and sequestration mechanisms.

In the present study, the maximum growth (1.202 abs) was obtained in BPT soaked RME compared to BPT boiled, ADT soaked and boiled RME (Fig.1 and 2). This may be due to the available nutrient concentration in the RME [13]. Among the six different microalgae strains tested, *Cosmarium* sp.,

showed higher growth rate than the *Chlorella vulgaris* (CV), *Scenedesmus product* (SPr), *Scenedesmus peccensis* (SPe), *Chlorella* sp., (CHL) and *Chloromonas* sp., (CHM) which might be due to the interaction effects of various nutrients and environmental factors of algal culture systems [14]. Similar results have been observed by early workers using microalgae [15-17].

Table 1: Physico-chemical characteristics of untreated ADT 43 soaked and boiled RME sample

Parameters	Soaked ADT 43 RME sample	Boiled ADT 43 RME sample
pH	6.5	8.8
Colour	Light brown	Light brown
Salinity (ppt)	2	2
Dissolved oxygen (mg L ⁻¹)	24.56	7.8
Temperature (°C)	25	27
Calcium (mg L ⁻¹)	32	8
Magnesium (mg L ⁻¹)	13.44	3.36
Phosphate (μmol/l)	10.9	0.36
Nitrate (μmol/l)	1.75	8.66
Ammonia (μmol/l)	12.69	6.54
Silicate (μmol/l)	13.69	16.3
Nitrite (μmol/l)	29.19	0.38
Total alkalinity	27	4

Table 2: Physico-chemical characteristics of untreated BPT soaked and boiled RME sample

Parameters	Soaked BPT RME sample	Boiled BPT RME sample
pH	7.5	7.06
Colour	Light brown	Light brown
Salinity (ppt)	2	2
Dissolved oxygen (mg L ⁻¹)	38	9.157
Temperature (°C)	28	25
Calcium (mg L ⁻¹)	20	12
Magnesium (mg L ⁻¹)	1.1	2.24
Phosphate (μmol/l)	58.6	2.38
Nitrate (μmol/l)	0	17.98
Ammonia (μmol/l)	12.6	9.18
Silicate (μmol/l)	28.4	4.318
Nitrite (μmol/l)	32.5	2.51
Total alkalinity	3	2

Fig. 1: Growth of microalgae in ADT boiled and soaked RME sample

Fig. 2: Growth of microalgae in BPT boiled and soaked RME sample

CONCLUSION

Microalgae are considered to be promising for future raw feedstock because of their potency to produce derivative products in biorefinery processes by utilizing effluent. The entire study advocates the suitability of rice mill effluent as a proficient growth medium for cultivation of microalgae

Cosmarium sp. This study concludes that the high concentration of nitrogen and phosphate present in rice mill waste water is a good nutrient sources for algal biomass production, which could be used for a range of purposes including biodiesel production and animal feed (or) in aquaculture. The results of this study also suggest that rice mill

effluent can be used for replacing synthetic media for cultivation of microalgae.

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