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A Review on Microphytes Biotechnology in a Wastewater Treatment

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Abstract: *The need for more efficient wastewater treatment requires technologically advanced major capital investments and maintenance costs. These advanced treatment systems are often out of reach for those in less developed or rural areas. However, an effective alternative, low energy input systems that utilize microphytes and the natural biological process of photosynthesis can drive efficient wastewater treatment systems. Microphytes play a vital role in the assimilation of pollutants in water systems. Although microphytes exist throughout natural water systems in various forms and concentrations, it is the mass culture (or the concentrated growth and management) of microphytesthat makes it viable for wastewater treatment. In wastewater treatment facilities, microphytes can be used to reduce the amount of chemicals needed to clean and purify water. The major advantage of microphytes waste water treatment technology has over conventional aerobic wastewater treatment systems is reduced cost due to the decrease in energy input.*

Keywords: *Microphytes, pollutants, biofuels, pathogens, photo bioreactor, dissolve oxygen.*

1. INTRODUCTION

Almost all the human activities can and do impact adversely upon the water. Water quality is influenced bymainly agricultural, municipal, industrial discharge etc. For agriculture, the key pollutants include nutrients, pesticides, sediment etc. There can be significant health hazards related to using untreated wastewater in agriculture. Wastewater from cities can contain a mixture of chemicals and biological pollutants [1]. In low income countries, there are often high levels of pathogens from excreta, while in emerging nations, where industrial development is outpacing environmental regulation; there is increasing risk from inorganic and organic chemicals. Today,1.2 billion people call Indian home, 377.1 million of whom who lives in cities. In the next 40 years, India's population is expected to grow by nearly half a billion. In the next two decades alone, 225 million more people will be living in Indian cities. The water required to serve this population today is 740bn cubic meters per year. By 2030, this number is expected to grow by approximately 200%, to nearly 1.5 ton cubic meters per year. The real challenge is to provide enough clean water to a rapidly growing global population. Our main focus for this work is to how we treat municipal wastewater by efficient technique and in a most economical way.

Microphytes are oxygen evolving photosynthesis microorganisms and commonly grow in a various aquatic environment, such as fresh and marine water, wastewater stream from a variety of wastewater source (e.g., agriculture run-off, concentrated animal feedoperations, and industrial and municipal wastewaters). Microphytes bacterial based biotechnology has received more and more attention in recent years, especially in the subtropical and tropical regions, as an alternative method of conventional multistep wastewater treatment processes [2]. Moreover, the microphytes biomass generated during wastewater treatment is regarded as a sustainable bio resource which could be used for producing biofuel, agriculture fertilizers or animal feeds.Wastewater borne microphytes bacterial culturecultivatedand trained through alternate mixing and non-mixing strategy was used to treat pretreated municipal wastewater. After one month of cultivation and training, the acclimatized microphytes bacterial system showed high carbon and nutrient removal capacity and good settleability within 20 minutes of sedimentation.

Microphytes biomass uptake was the main removal mechanism of nitrogen and phosphorus. The biomass productivity, nitrogen and phosphorus accumulation in biomass during the wastewater treatment process were investigated. The characterizations of the microbial consortium composition in the enriched microphytes bacterial system provide new insights in this research field. Microphytes growth promoting bacteria (MGPB) used as inoculants in wastewater treatment, both for control of phytopathogens and plant growth promotion [3]. Bacteria of the genus Azospirillum are well known as MGPB for numerouscrop plants [4]. A combination of microphytes (Chlorella vulgaris or C. sorokiniana) and a microphytes growth promoting bacterium (MGPB, Azospirillumbrasilense strain Cd), co-immobilized in small

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alginate beads, was developed to remove nutrients (P and N) from municipal wastewater. Sometime aerobic activated sludge, which already showed good settleability was used as bacterial inoculum to enhance the wastewater treatment performance and biomass settleability of microphytes bacterial culture. There was no significant effect of the inoculation ratios on the chemical oxygen demand (COD) removal. Under illuminated conditions, microphytes uptake nutrients and produce O_2 that may be used as an electron acceptor by aerobic bacteria to degrade organic matter [5,6].Microphytesalso consume the CO_2 released from bacterial respiration to complete the photosynthetic cycle, which contributes to greenhouse gas abatement. In wastewater fertilized systems, the role of the microphytes include O_2 supply, nutrient uptake and adsorption of heavy metals.

2. MUNICIPAL TREATMENTPLANTS

A typical municipal sewage treatment plant in an industrialized country may include primary treatment to remove solid material; secondary treatmentto remove dissolved and suspended organic material as well as the nutrients nitrogen and phosphorus, and sometimes but not always disinfection to kill disease causing micro-organisms. Larger municipalities often include factories discharging industrial wastewater into the municipal sewer system. Secondary treatment is designed to substantially degrade the biological content of the sewage which are derived from human waste, food waste, soaps and detergent. Tertiary treatment is a term applied to polishing methods used following a traditional sewage treatment sequence [7]. Tertiary treatment is being increasingly applied in industrialized countries and most common technologies are micro filtration or synthetic membrane. After membrane filtration, the treated wastewater is nearly indistinguishable from waters of natural origin of drinking quality (without its minerals).

2.1 MUNICIPAL WASTEWATER TREATMENT WITH MICROPHYTES

Municipal wastewater treatment with microphytesbased technology offers acost efficient and environmental friendly alternative to conventional treatmentprocesses. In this system, microphytes assimilate nutrients in wastewater for theirgrowth, generate oxygen through photosynthesis thus offering a low cost aerobicenvironment, absorb heavy metals and indirectly remove pathogens and viruses byincreasing pH, temperature and dissolved oxygen concentration duringphotoautotrophic metabolism .So far,microphytes algaebased technology is used for pretreated wastewater treatment, tertiary treatmentand other advanced treatment [8].

2.2 PRETREATED MUNICIPALWASTEWATER TREATMENT WITH MICOPHYTES

When using microphytesbacterial culture for pretreated municipal wastewater treatment, organic matter is oxidized by heterotrophic bacteria into CO_2. Under the illuminated condition, microphytes fix the generated CO_2, assimilate the nutrients (NH_4^+, NO_3^- and PO_4^{3-}) in the wastewater and simultaneously produce O_2 through their photosynthesis, which is required by aerobic bacteria as electron acceptor. In conventional wastewater treatment plants, the oxygen supply accounts for more than 56% of the total energy cost. While, the energy consumption of microphytes based technology is greatly reduced as microphytes provide a low cost aerobic condition for aerobic bacteria [9]. In addition, heterotrophic algae could also contribute to the organic carbon removal in the dark.

2.3 TERTIARY TREATMENT WITH MICROPHYTES BASED BIOTECHNOLOGY

Microphytes based technology has also been applied for tertiary treatment especiallyfor further nitrogen and phosphorus removal, through two possible mechanisms: biotic removal through biomass assimilation and abiotic removal induced by theincrease in pH through microalgal photosynthesis [10]. Microphytes require high amounts of nitrogen and phosphorusfor protein (45-60% of microphytes dry weight), nucleic acids and phospholipidssynthesis. The nitrogen assimilation could be increased afterpretreatment of microphytes by starvation.In addition, as thecontent of organic carbon in tertiary influent is low, the CO_2 for microalgalphotosynthesis is mainly in forms of inorganic carbon from the air. It is reported thatpH will increase in reactors with high inorganic/organic carbon during the microphytesphotosynthesis. Besides, the microphytes photosynthetic growth wouldalso cause the increase in ph. The elevated pH in the system would lead to ammoniavolatilization and phosphorus precipitation, which contributes to the overall nutrientremoval efficiency.

2.4 REMOVAL OF BOD AND COD

Research studies by Algaetech have shown that some microphytes strains have the ability to reduce BOD by 96% and COD by 93%, while turbidity and heavy metals are kept at acceptable range. Microphytes are natural consumer of carbon dioxide and emitter of oxygen, our environment is kept clean throughout the process. Therefore, wastewater which is readily available in any regions can be a mean for CO_2/O_2 balance, and also converted to one of the world's most wanted commodity – fuel, while maintaining food sustainability. If biological oxidation is employed the test is termed the Biochemical Oxygen Demand (BOD), whereas for chemical oxidation, the test is termed Chemical Oxygen Demand (COD). In other words, BOD exploits the ability of microorganisms to oxidise organic material to carbon dioxide and water using molecular oxygen as an oxidizing agent. Therefore, biochemical oxygen demand is a measure of the respiratory demand of bacteria metabolizing the organic matter present in wastewater. Excess BOD can deplete the dissolved oxygen of receiving water leading to fish kills and anaerobiosis, hence its removal is a primary aim of wastewater treatment.

2.5 CONVERSION INTO BIOMASS

The capacity of plants to contribute to biomass production is much lower than that required; thus, other alternatives such as microphytes have been proposed. Microphytes can grow faster than plants, do not require fertile land or useful water, reach solar energy utilization efficiencies up to 5 %, and are able to use direct flue gases as their carbon source. The production of 1 tonne of microphytes biomass requires a minimum consumption of 1.8 tonne of CO_2, so CO_2 can be captured from flue gases and transformed into biomass, valuable biofuel. In the same way that N and P could potentially be obtained from waste streams such as municipal wastewater, it is conceivable that the CO_2 required to support high rates of microphytes growth and productivity in mass culture could be supplied by the flue gas emissions from heavy industry. This application, sometimes referred to as 'CO_2biomitigation' is seen as a potential strategy to capture CO_2 from municipal waste sources, before it has a chance to escape into the atmosphere[11,12].The amount of microphytes produced worldwide (5 kilotonne/year) is almost nothing compared to worldwide CO_2 emissions (20 Gross tonnage/year). Therefore, in order to contribute to a significant reduction in CO_2 emissions, a major scaling up of current technologies is needed to be able to produce large amounts of microphytes biomass. On the other hand, the microphytes biomass produced today is mainly used for human or animal consumption, its price being high (10–300€/kg), but the size of these markets is small (10–50 kilotonne/year); consequently, large amounts of biomass entering these markets would reduce the price [13, 14].

3. FACTORS AFFECTING MICROPHYTES BACTERIAL SYSTEM

3.1TEMPERATURE

The optimum temperature for microphytes growth usually varies with the species and also depends on the acclimated environment. Below the optimum temperature, microphytes growth rates would increase with an increase of temperature [15]. Above the optimum temperature, microphytes growth rates would decrease with increasing temperature. Generally, temperatures higher than 35 ^{0}C are lethal for some algal species while temperatures below 16 °C would slow down microphytes growth unless isolated from extreme environments.

3.2 PH

PH does not only affect the growth of microphytes but also modify the $CO_2/HCO_3^-/CO_3^{2-/}NH_3/NH_4^+$equilibria and phosphorus and heavy metal availability. Furthermore, pH is important for processes of abotic nutrient removal as at a pH of 9 to 11 some of the inlet nitrogen and phosphorus are removed via ammonia stripping and orthophosphate precipitation [16]. Changes in pH levels influence the bacterial activity in microphytes bacteria culture. In addition, the increase in pH would promote flocculation thus offering benefits for the biomass separation. A high inorganic/organic carbon ratio would lead to high pH, and vice versa.

3.3 DISSOLVED OXYGEN (DO)

Dissolved oxygen concentration increases during microphytes photosynthesis, as the growth of 1 g of microalgal cells is accompanied by the release of 1.587 g of dissolved molecular oxygen [16]. The generated oxygen would be consumed immediately by heterotrophic bacteria or heterotrophic microphytes for organic carbon degradation and the finial DO levels are the combined results of oxygen generation and consumption. For instance, the DO concentration was as low as approximately 0 mg/l after starting the batch test indicating that the generated oxygen was completely used for organic carbon mineralization. DO increase immediately to around 5 mg/l as soon as the organic carbon source was eliminated. Above 15 mg/l of DO was observed after beginning the test and this high DO value remains stable till the end of the test for tertiary wastewater treatment.

3.4 MIXING

Mixing may affect the microphytes growth in several important ways. First, it provides the microalgal cells intermittent contact with the light, thereby increasing the available light utilization efficiency. Second, it may enhance the contact between the microalgal cells and nutrients and limits nutritional gradients in the cultivation medium [16]. Third, it also helps the CO_2 transfer and O_2 release into atmosphere. Fourth, it keeps all the microalgal cells suspension and avoids the formation of anaerobic zones and further anaerobic decomposition.

3.5 MICROPHYTES SPECIES SELECTION

Many different microphytes species have been selected and tested for their potentials in wastewater treatment performance, microphytes biomass production or lipid content under various experimental conditions. Among the different microphytes, Chlorella, Chlamydomonas and Nitzschia seem to be the preferred species of microphytes due to their good tolerance of different environmental conditions. Oswald et al (2003) showed that Chlorella, Scenedesmusand Micractinium were dominant algal species in algae wastewater treatment ponds, and species of Euglena, Chlamydomonas and Oscillatoriamay occur in ponds with excessive loadings or long residence times, indicating that these six species may play important roles during wastewater treatment. Especially, Chlorella is viewed as indigenous species usually found in wastewater treatment plants.

4. OTHER USES OF MICROPHYTES

4.1 BIOFUEL PRODUCTION

Microphytes offer great potential as a sustainable feedstock for the production of third generation biofuels. Microphytes production in wastewater treatment is potentially an economically viable feedstock for biofuel production. However, increasing biomass is still regarded a top priority to make microphytes biofuel a commercial reality[17, 18,19]. Microphytes light absorption and photosynthesis are limited in wastewater pond and the improvement of these will undoubtedly increase biomass yield. The technical feasibility of many algae production technologies has been extensively investigated and demonstrated. However, the economic viability and environmental sustainability remain the key obstacles to the commercialization of these technologies.Microphytes grow quickly and contain high oil content compared with terrestrial crops, which take a season to grow and only contain a maximum of about 5 percent dry weight of oil. They commonly double in size every 24 hours. During the peak growth phase, some microphytes can double every three and one-half hours. After separation of microphytes, the purified water can be reused for industrial purposes while the microphytes biomass can be harvested for biofuel, plus, the extracted residual biomass with high protein and carbohydrates is suitable for animal and aquaculture feed.

TABLE 1: Oil content ofmicrophytes

Microphytes	Oil content (% dry weight)
Botryococcusbraunii	25-75
Chlorella sp.	28-32
Crypthecodiniumcohnii	20
Cylindrotheca sp.	16-37
Nitzschia sp.	45-47
Phaeodactylumtricornutum	20-30
Schizochytrium sp.	50-77
Tetraselmissuecia	15-23

TABLE 2: Oil yields based on crop type.

Crop	Oil yield (gallons/acre)
Corn	18
Soybeans	48
Canola	127
Jatropha	202
Coconut	287
Oil Palm	636
Microphytes	6283-14641

4.2 BIO-HYDROGEN

Microphytes for photobiological hydrogen production from water are being developed into a promising and a potentially emission free fuel stream for the future, which could also be coupled to atmospheric CO_2sequestration. [20,21,22]The bio-hydrogen process is attractive in that it uses sunlight to convert water to hydrogen and oxygen, which are released in a two phase process through the following reactions:

$H_2O \rightarrow 2H^+ + 2e^- + {}^1/_2O_2$

$2H^+ + 2e^- \rightarrow H_2$

The first reaction occurs in all oxygenic photosynthetic organisms while the second reaction is mediated by special iron containing chloroplast-hydrogenase enzymes and is restricted to a more select group of microphytes [23,24]. Cyanobacteria also have the ability to produce H_2 from water but use an alternative biochemical process. Under normal light and aerobic conditions, H^+ and e^- from the water-splitting reaction of photosynthesis are used for synthesis of Adenosine Triphosphate (ATP) and Nicotinamide adenine dinucleotide phosphate (NADPH). The second reaction occurs under anaerobic conditions. In the absence of O_2 both ATP production by oxidative phosphorylation, and the formation of NADH/NADPH are inhibited [25]. Under these conditions, certain microphytes, such as Creinhardtii reroute the energy stored in carbohydrates such as starch to the chloroplast hydrogenase [22] to facilitate ATP production via photophosphorylation and to keep the electron transport chain from over reduction. Thus, hydrogenase together with different fermentative processes essentially acts as a proton/electron release valve by recombining protons from the medium and e- from reduced ferredoxin to produce hydrogen gas that is excreted from the cell [26].

5. CULTIVATION FACILITY

Reactors used for mass cultivation of phototrophic microorganisms (including microbial, algal or plant cells) in a photobiological reaction are termed Photobioreactors (PBR). Depending on whether or not exposure to the air, PBR for microphytes cultivation can be simply classified as open systems or closed systems. The commonly used open systems in wastewater treatment plants include lagoon, high stabilization pond, aerated ponds, raceway pond, multilayer pond like bioreactor, etc. The most prominent features of open pond system include simple construction, low cost and simple operation [27]. However, the disadvantages of such system are also obvious, such as large occupation area, not stable culture system, difficult to control, easy to get polluted, water evaporation loss issue and the fact that light intensity decays rapidly with medium depth. None the lessly, from the perspective of economic viability, advanced raceway pond with paddle wheel is considered as the most promising culture system for low cost treatment of different types of wastewaters generated from sewage, industry and agriculture as well as biofuels feedstock accumulation. Besides saving water, energy and chemicals, closed bioreactors have many other advantages which are increasingly making them the reactor of choice for biofuel production, as their costs are reduced. The most important among these aspects is that they support up to fivefold higher productivity with respect to reactor volume and consequently have a smaller "footprint" on a yield basis[28]. However, there is still a gap between designing a high end reactor which meets all demands of the algal cells on the one hand, and a cheap reactor on the other hand, which enhances the economic viability of the process[29]. To increase process efficienciesphotobioreactor have to be designed to distribute light over a large surface area in order to provide moderate light intensities for the cells [26].

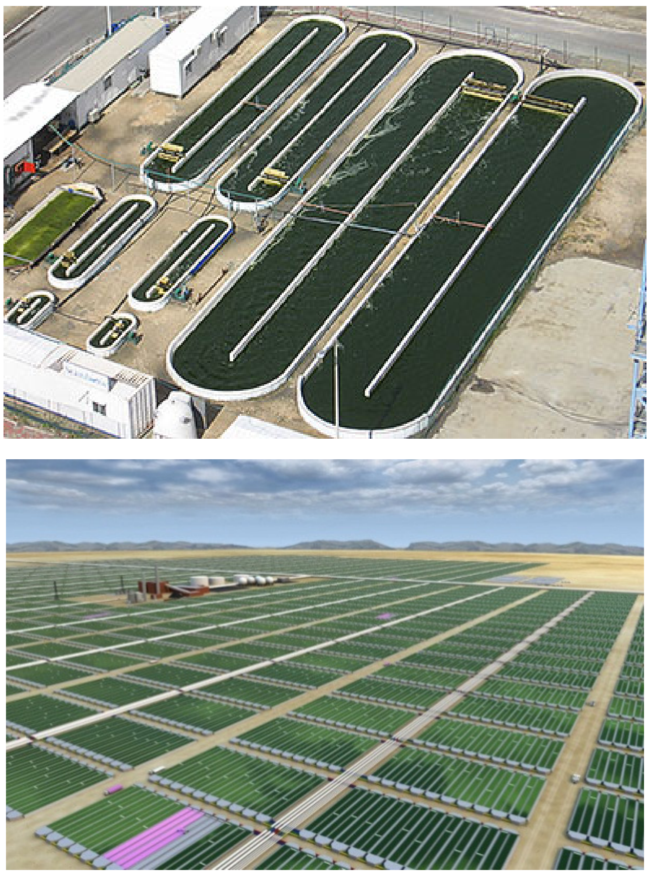

Fig. 1.Open and close pond microphytes cultivation facility.

6. HARVESTING

Separating the microphytesfrom water remains a major hurdle to industrial scale processing partly because of the small size of the microphytes cells. Various methods are currently used for harvesting microphytes, which includes chemical based, mechanical based, biological based and to a lesser extent, electrical based operations However, various combinations or sequence of these methods are also commonly in use [30,31]. The cell size of microphytes is very small. Therefore, chemical flocculation is often performed as a pretreatment to increase the particle size of microphytes before using another method such as flotation to harvest the microphytes. In mechanical based process, centrifugation process, which is the most reliable and rapid method, is used for recovering suspended microphytes.

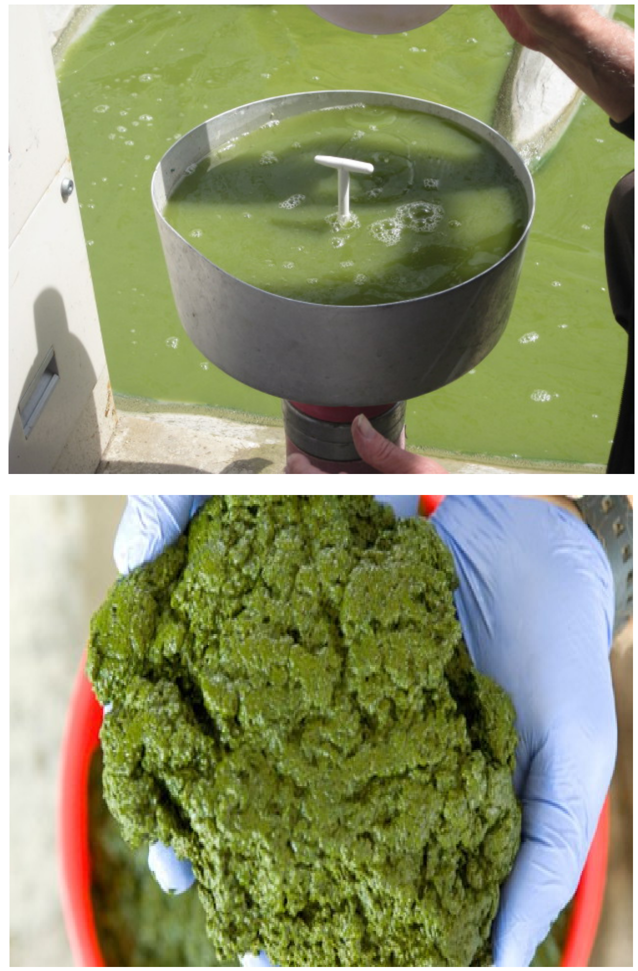

Fig. 2. Microphytes harvesting by centrifugation process.

7. ADVANTAGES

- Microphytes are considered as a great model of photosynthetic organisms and have a higher carbon capture efficiency compared to other photosynthetic plant feed stock.
- The above studies have reported that microphytes have a great potential for the removal of nitrogen and phosphorus.
- Microphytes can take up kinks of inorganic or organic nutrients from wastewater, and this capacity could be explored in commercial water treatment.
- Due to the small size of microphytes cellsand the dilute nature of microphytes cultures, the main hindrance to microalgal wastewater treatment is the removal of

biomass from the treated effluent under free-cell cultivation conditions.

- The microphytes biomass generated during wastewater treatment is regarded as a sustainable bio resource which could be used for producing biofuel, agricultural fertilizers or animal feeds.

8. CONCLUSIONS

Microphytes, managed in a controlled environment, are a proven low initial capital cost, low operational cost supplement or alternative to mechanically aerated wastewater treatment systems. The microphytes are grown in ponds and/or photo bioreactor, which in combination with sufficient retention times, suitable pH levels, dissolved gases and sunlight, the microphytes are able to bio remediate the wastewater effluent.Microphytesbased wastewater treatment systems are particularly advantageous in low land-cost areas where sunlight and warm temperatures are plentiful. The multiple-function of microphytes is a new approach and still needs more investigation. Although this technology is attractive, a number of obstacles need to be solved before large scale applications.

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