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Research Article

In Vitro Antimicrobial Activity Along with Biomass Production in Wastewater by Cyanobacteria *Spirulina platensis*

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ABSTRACT

With the exponentially increasing population pressure and huge amount of wastewater thus generated, biological wastewater treatment systems has drawn the interests of scientists in recent years. Use of cyanobacteria in wastewater treatment could prove beneficial as they would also increase oxygenation and mineralization. In present study i*n vitro* antimicrobial activity along with biomass production in domestic wastewater by Cyanobacteria *Spirulina platensis* was evaluated. *Spirulina platensis* removed coliforms and faecal coliforms considerably in respect with control. Cyanobacteria cause diurnal variation of pH, alkaline pH and temperature increase as a result of conversion of light energy to heat energy and antibacterial exudates which help in pathogenic bacteria reduction. The rate of growth was very high during the first week but almost stagnant after three weeks of growth, seemingly because of nutrient depletion. The extracts of the cyanobacterial strain, especially ethanolic extract were effective against *Escherichia coli, Staphylococcus aureus, Salmonella enterica* and fungus *Aspergillus niger.*

Keywords: Cyanobacteria, *Spirulina platensis*, wastewater, biomass, antibactetrial.

INTRODUCTION

There is a delicate balance in the environment, incorporating all the biotic and abiotic factors. Given the vast and largely untapped diversity of microorganisms and the versatility of their catabolic processes, it seems probable that many, if not all of these pollutants could be amenable to bioremediation[1].

Domestic sewage contains high amounts of biodegradable organic matter, inorganic nutrients and huge amount of microorganisms. Such wastewater, when discharged into water courses without proper treatment, is not only unfit for irrigation or aquaculture but can deplete dissolved oxygen from the receiving water and pollute surface and ground waters, thus rendering these waters too unfit for uses like drinking and irrigation[2]. Govindan (1989)showed that sewage of Indian domestic area contains high amounts of such pollutants and nutrients[3].

With the exponentially increasing population pressure and huge amount of wastewater thus generated, biological wastewater treatment systems has drawn the interests of scientists in recent years[1, 4]. Biological treatment is a cheaper, more efficient and sustainable choice than conventional chemical wastewater treatment and recycling [5 6]. Cyanobacteria are perfect organisms for biological treatment as they are photoautotrophic and inexpensive and easy enough to maintain [7]. Cyanobacteria may release several antimicrobial exudates like bacteriocins, polyphenols and terpenoids which in turn may inhibit other members of microbial community [8-12]. Cyanobacterial biomass is also economically useful for different byproducts like amino acids, proteins

and vitamins [10]. Wastewater can thus be treated in waste stabilization ponds by cyanobacteria, alone or in symbiosis with heterotrophic bacteria, for reuse of treated wastewater in aquaculture or agriculture and for production of microbial biomass for beneficial uses [13].

Different species of cyanobacteria are normally found in wastewater [2, 14]. Though the removal of nutrients like nitrogenous and phosphorus compounds, which may lead to cultural eutrophication, has been studied by several workers, prospect of removal of pathogenic bacteria by living organisms has been neglected [4, 15-17]. Few studies had been done in natural wastewater treatment systems [18, 19]. Recently workers are searching for strains of cyanobacteria with high biomass yields and high utilization potential for mass production in wastewater [17]. The cyanobacteria produced in the wastewater could be used as a source of low cost but high food-value protein [20]. In present study i*n vitro* antimicrobial activity along with biomass production in domestic wastewater by Cyanobacteria *Spirulina platensis* was evaluated.

MATERIALS AND METHOD

Sampling and Preservation

Untreated wastewater samples were collected from the inlet point of Kalyani Sewage treatment plant situated in the satellite township of Kalyani, West Bengal. This wastewater is generally domestic in nature. The plant gets about 10-12 million gallons wastewater per day. Samples were collected in polyethylene wide-mouth bottles of minimum sample size 1l. It was important to avoid surface materials during sampling. Sterilized glass bottles were used for microbial analyses [4, 21]. Samples were taken to laboratory keeping it protected from direct sunlight and heat and analyzed as early as possible. Refrigeration at 4ºC and addition of preservatives, if necessary, were done according to APHA [21].

Microbial analyses

All physico-chemical analyses and Heterotrophic plate counts were done by pour plate method after incubation for 48 hours in plate count agar [21]. Samples were serial-diluted with sterilized distilled water for getting appropriate counts. All microbiological operations were done aseptically. Total and Faecal coliform counts were done according to multiple tube fermentation method of APHA [21].

Organisms used

One environmental isolate *Spirulina platensis* (isolated from sewage) was used in this study. It was maintained in Zarrouk's medium at 28°C with 500 lux light intensity [22].

Pathogenic bacteria removal by cyanobacterial culture from wastewater

500ml wastewater samples in 1l Erlen-meyer flasks were inoculated with 2-3 ml of fresh cultures of different species (to reach an initial inoculum concentration of about 0.5 μg chlorophyll-a ml^{-1} sample) and incubated at the cultural conditions given above. Respective negative control sets were run simultaneously. Another set of controls comprising inoculated media (Zarrouk's) were also run. To study the effect of cyanobacteria like *Spirulina* Sp. on pathogenic bacteria, raw unsterilized wastewater was incubated with *Spirulina* sp. for 3, 6 and 9 days. After proper incubation, pathogenic bacteria were enumerated [21].

Biochemical estimation of biomass

After the necessary incubation period, microbial cultures were harvested and the chlorophyll-a content [23] and protein content [24] were estimated.

Aqueous and ethanolic extracts

For in vitro antimicrobial assay microbial strains used and method of preparation of aqueous and ethanolic are as described by Das [25]. Aqueous extract of each part was prepared by boiling 5 g of dried cyanobacterial powder in 100ml sterile distilled water over moderate flame for 20 min. The aqueous extract was cooled, double filtered through Whatman No.1 filter paper and then kept in sterile screw capped glass vials at 4ºC. Five gram of powder sample was crushed in ethanol for 48 hours at 24ºC with stirring. The extracts were centrifuged and filtered through Whatman No.1 filter paper and evaporated using vacuum rotary evaporator to near dryness and stored in glass vials in dark at 4ºC.

Disc diffusion bioassay

The disc diffusion test was carried out as described by Jorgensen et al. [35]. A 0.5 ml standardized inoculum suspension of each bacterial strain was spread on TSA plates with a sterile bent glass rod spreader. Sterile 6-mm Whatman no.1 filter paper discs were aseptically placed on plates. Sample decoctions or extracts of standard concentrations (10 mg dry weight) were aseptically poured on the discs along with sterile double distilled water or 10% DMSO as negative and ampicillin as positive controls. Plates were allowed to stand for 30 minutes at room temperature prior to incubation at 35-37 ºC for 24 hours. The inhibition zone diameters were

measured three times and means were represented [26].

RESULTS AND DISCUSSION

The nature of domestic wastewater (settled) collected from Kalyani Sewage Treatment Plant inlet point was shown in Table 1. The pH, which was almost neutral at the time of collection, turned alkaline (pH 8.8) after sterilization for the purpose of experiment. Domestic sewage contains high amounts of biodegradable organic matter and inorganic nutrients. The nutrient levels of the raw wastewater were found to be moderate in nature. Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) levels were also high for further reuse in agriculture or aquaculture directly.

After incubation with *Spirulina platensis* for 3, 6 and 9 days, total coliforms and faecal coliforms of wastewater were gradually reduced (Table 2). *Spirulina platensis* removed coliforms and faecal coliforms considerably in respect with control. This phenomenon was probably due to adverse environmental conditions formed by cyanobacteria like – diurnal variation of pH, alkaline pH and temperature increase as a result of conversion of light energy to heat energy and antibacterial exudates [27].

Chlorophyll and Protein content of the strain in wastewater increased uniformly (Table 3). Rate of growth was higher in the 1st week. The biochemical changes in wastewater were estimated in terms of chlorophyll-a and protein-contents. The rate of growth was very high during the first week but later decreased. Growth was almost stagnant after three weeks of growth, seemingly because of nutrient depletion. In most cases growth was optimum on second week after which it had decreased to some extent. The protein content (In first two weeks) was found to be comparable to that of organism grown in appropriate culture media without addition of extra carbon-source or minerals. Bhagwat and Apte also noticed similar trend in *Anabaena* sp. [28].

The extracts of the cyanobacterial strain were effective against *Escherichia coli, Staphylococcus aureus, Salmonella enterica* and fungus *Aspergillus niger* (Table 4). Highest DIZ was shown against *Escherichia coli* treated with ehanolic extract. *Serratia marcescens* is the most resistant strain tested against these extracts. Ethanolic extract was more effective than aqueous extract. The antimicrobial activity of of *S. platensis* was assumed to be due to the presence of organic compounds like γ- Linolenic acid, hexadecanoic acid, methyl ester etc. [29, 30]. [Demule et al. (1996); Kumar et al. (2011)].

CONCLUSION

From the above studies it may be concluded that the wastewater concerned is suitable for mass-culture of cyanobacteria which in turn, can sufficiently reduce pathogenic microbial levels in sewage. Economically significant protein-rich strain *Spirulina platensis* was excellent for removing pathogenic bacteria from unsterilized wastewater. In such a process wastewater might be removed of nutrients and pathogens and simultaneously cyanobacterial biomass could be developed.

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Table 1
Physicochemical parameters of raw wastewater concerned.

Temperature (°C)	30.6±2.3
pH (Before sterilization)	7.3±0.2
pH (After sterilization)	8.8±0.3
BOD5 (mg l-1 O2)	93.4±4.1
COD (mg l-1 O2)	165.0 ±11.5
Nitrate-content (mg l-1)	0.41±0.06
Ammonia (mg l-1)	12.8±1.7
Total Phosphorus (mg l-1)	2.55±0.24
Total coliform count (per 100ml)	8.1±0.3x109
Faecal coliform count (per 100ml)	4.5±0.2x108

Table 2
Change of total and faecal coliform counts of wastewater under incubation with *Spirulina* platensis

Days	Incubation with *Spirulina platensis* I		Control	
	Total coliforms	Faecal coliforms	Total coliforms	Faecal coliforms
0	$7.8x\ 10^9$	$4.6x\ 10^8$	$7.8x\ 10^9$	$4.6x\ 10^8$
3	$3.5x\ 10^8$	$7.2x\ 10^7$	$5.2x\ 10^9$	$2.3x\ 10^8$
6	$2.1x\ 10^7$	$8.7x\ 10^6$	$4.1x\ 10^8$	$6.6x\ 10^7$
9	$9.4x\ 10^5$	$6.3x\ 10^4$	$3.2x\ 10^7$	$8.1x\ 10^6$

Table 3
Biomass yield in terms of Chlorophyll-a content and Total protein content of Cyanobacteria in wastewater

Organisms	Incubation			
	Day 0	Day 7	Day 14	Day 21
Weekly Chlorophyll-a content (µg/ml.)	0.5±0.02	3.83±0.15	5.21±0.18	6.03±0.16
Control	0.5±0.02	3.78±0.18	5.4±0.32	6.5±0.5
Total protein content (µg/ml.)	35±2.2	267±8.2	352±15.4	369±12.6
Control	35±2.2	258±10.5	370±10.5	427±12.2

Table 4
Antibacterial activities, indicated by diameter of inhibition zone (DIZ, mm, for 10 mg dry wt./ disc, Mean±SD) of *Spirulina platensis* against the micro-organisms
[- means <7mm DIZ i.e DIZ of negative control]

Microorganisms	*Spirulina platensis*	
	Aqueous	Ethanolic
E.coli	9±1	11±1.527
S. aureus	8±0.577	9±0.577
S. enterica	10±1	10±1.527
B. cereus	10±1	9±1
S. marcescens	-	-
L. brevis	8±1	10±1
A.niger	8±1.527	9±1.527

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