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Biocalcification Mediated Remediation of Calcium Rich Ossein Effluent by Filamentous Marine Cyanobacteria

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Abstract

Biocalcification of excess calcium catalyzed by the growth of the selected filamentous marine cyanobacteria exemplifies potential mechanism towards bioremediation of ossein effluent released during acidification of cattle bones in a gelatin production system. Two filamentous marine cyanobacteria *Oscillatoria willei* BDU130791 and *Phormidium valderianum* BDU20041 were tested for their ability to grow and aid in bio-calcite formation under laboratory conditions. Calcite formation, has been demonstrated in three types of effluent collected at different stages namely, di-calcium phosphate (DCP), high total dissolved solids (HTDS) and low total dissolved solids (LTDS). Biomass induction and calcite formation, were observed in effluents with seawater dilution in the ratio 1:1 amended with fertilizer grade urea and superphosphate. Biomass productivity of 0.15 g L^{-1} d^{-1} and 0.06 g L^{-1} d^{-1} were obtained in LTDS and HTDS effluent by *O. willei* BDU130791 and *P. valderianum* BDU20041 respectively with concomitant nutrient removal rates at the end of seventh day coupled with simultaneous increase in dissolved oxygen (DO) and calcite formation. Biocalcite formed by both the marine cyanobacteria was further characterized by scanning electron microscopy coupled fourier transform IR and electron dispersive spectral (EDS) analysis. The study for the first time has unraveled the potentials of *O. willei* BDU*130791* and *P. valderianum* BDU20041 to remediate the ossein effluent by calcite formation.

Keywords: Ossein; Cyanobacteria; Bioremediation; Calcification

Introduction

Ossein (decalcified bone) is the chief organic substance of the animal bone tissue obtained as a residue in the clarification process of gelatin production system. The huge volume, strong odor and the high organic and inorganic contents are of major concerns in disposal of the ossein effluent. Decalcification of cattle bones generates huge volumes of calcium opulent effluent namely (i) di-calcium phosphate (DCP) rich-immediately released upon acidification of the bones, (ii) high total dissolved solids (HTDS)-released on heat treatment of the decalcified bones and (iii) low total dissolved solids (LTDS) effluent-discharged after final washing and settlement of ossein.

The hardness of the ossein effluent is primarily due to its high calcium content that has enticed the attention for bioremediation. Cyanobacteria, the primordial photosynthetic organisms have an edge over the other heterotrophic bacteria since they evolve oxygen with tropical independence to carbon and nitrogen [1] and can thus effectively grow in organic rich effluent and in extreme conditions. Among cyanobacteria, marine forms have varied applications because of their ability to thrive in wide range of environmental regimes including the hazardous habitats. Marine cyanobacteria have been reported to degrade a number of effluents and toxic substances namely phenols [2], tannins [3], pesticides [4], diazo dyes [5], melanoidin [6] and adsorption of heavy metal and radionuclides [7].

The calcium richness is one of the prime factors because of which other organisms are unable to grow and remediate the ossein effluent wherein, calcium precipitation is an active process in cyanobacteria [8]. Carbon concentrating mechanism (CCM) of marine and freshwater cyanobacteria has been well explored and these organisms are known to fix co_2 as $caco_3$ [9]. Biologically catalyzed calcium carbonate precipitation represents an economically feasible approach for remediation coupled with carbon mitigation and biomass utilization in an industrial view point [10]. Hence an attempt was made to evaluate the biocalcification potential of selected non-heterocystous filamentous marine cyanobacteria and to optimize the growth conditions towards the remediation of ossein effluent.

Materials and Methods

Two filamentous marine cyanobacteria *Osillatoria willei* BDU130791 and *Phormidium valderianum* BDU20041 were tested for their growth in ossein effluent. The growth profile, nutrient removal rates and the calcifying ability of the strains were further evaluated and the biocalcites formed were characterized by scanning electron microscopic, electron dispersive and fourier transform infra-red spectroscopic techniques.

Source of the effluent

Calcium rich ossein effluent was collected at three different clarification stages from the gelatin manufacturing industry, Pioneer Jellice Industries, Cuddalore, Tamil Nadu, India. Three effluents namely dicalcium phosphate, (DCP), high total dissolved solids (HTDS), low total dissolved solids (LTDS) have been used for the present study. The effluent differed in amounts of the total dissolved solids (TDS), hence named as high TDS (HTDS) and low TDS (LTDS). The collected effluents are stored in black plastic cans at 4°C to avoid microbial growth till use. Autoclaved effluent was used for the experiments.

Strain selection and maintenance

Two filamentous non-heterocystous marine cyanobacteria,

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Oscillatoria willei BDU130791 and *Phormidium valderianum* BDU20041 of the order *Nostocales* were obtained from the culture collection of National Facility for Marine Cyanobacteria, Bharathidasan University, Tiruchirappalli based on our earlier studies on bioremediation [2,3,5,6]. The cultures were maintained in ASN III medium [11] under continuous white fluorescent light at an intensity of 20 µmol photon m^{-2} s^{-1} at 25 ± 2°C in controlled culture room. The chosen organisms were grown in effluent diluted with sea water (1:1. 2:1 and 3:1), amended with fertilizer grade nutrient enrichments (urea and superphosphate) as nitrogen and phosphorous sources.

Biomass determination

The end point growth of the selected strains in effluent sea water dilutions were estimated by determining its chlorophyll *a* content and dried biomass at the end of 7 days. Chlorophyll *a* was extracted with methanol and estimated by the extinction co-efficient given by mac kinney, [12]. The dry cell weight (g L^{-1}) was calculated by quickly washing the pellet thrice with distilled water and dried at 60°C. The dried biomass was weighed until at least two concordant values. The biomass productivity (g L^{-1} d^{-1}) was estimated according to the following equation [13]

$$P=X_1-X_0/T_1-T_0$$

Specific growth rate was measured as per the equation [14].

$$M=\ln(X_1/X_0)/T_1-T_0$$

X_1 and X_0 are biomass concentration at T_1 and T_0 days respectively.

Analysis of physico chemical properties

The physico chemical parameters of the three types of effluents were analyzed on every alternative day during the seven days experimental period. The un-inoculated effluent served as control. Upon centrifugation of the effluent grown cyanobacterial culture, the clear supernatant was used for the analysis.

The total solids (TS), total suspended solids (TSS) and total dissolved solids (TDS) were evaluated according to the standard methods [15] by drying the sample at 100°C using pre weighed crucibles. The nutrient removal by marine cyanobacteria from the effluents were evaluated by determining the amount of dissolved oxygen (DO), total Kjeldahl nitrogen (TKN), nitrate, nitrite, ammonia, total phosphorus, inorganic phosphorus, calcium and magnesium by following the standard methods for the examination of water and wastewater [15].

Calcification - FT-IR spectral studies

Fourier Transform Infrared (FT-IR) spectrum of the dried biomass of *O. willei* BDU130791 and *P. valderianum* BDU20041 grown in effluent on the seventh day. The organisms grown in ASN III served as control. The ASN and effluent grown cultures were dried at 60°C to remove moisture content and were–analyzed using Perkin Elmer Spectrum Gx FTIR (U.S.A) at 400-4000 cm^{-1} with a resolution of 1 cm^{-1}. Analytical-Grade Kbr was used as the dispersant.

Structural elucidation and characterization of the bio-calcite-SEM EDS analysis

The seven day old effluent grown culture morphology and of the calcite deposits on the treated biomass and the precipitated crystals were observed under a vega3 TESCAN scanning electron microscope (SEM) after gold sputtering. The elemental composition was studied by energy dispersion spectroscopy (EDS) using a qualitative and semi-quantitaive oxford edax spectrophotometer.

Statistical analysis

Each measurement was done in triplicate and the mean and

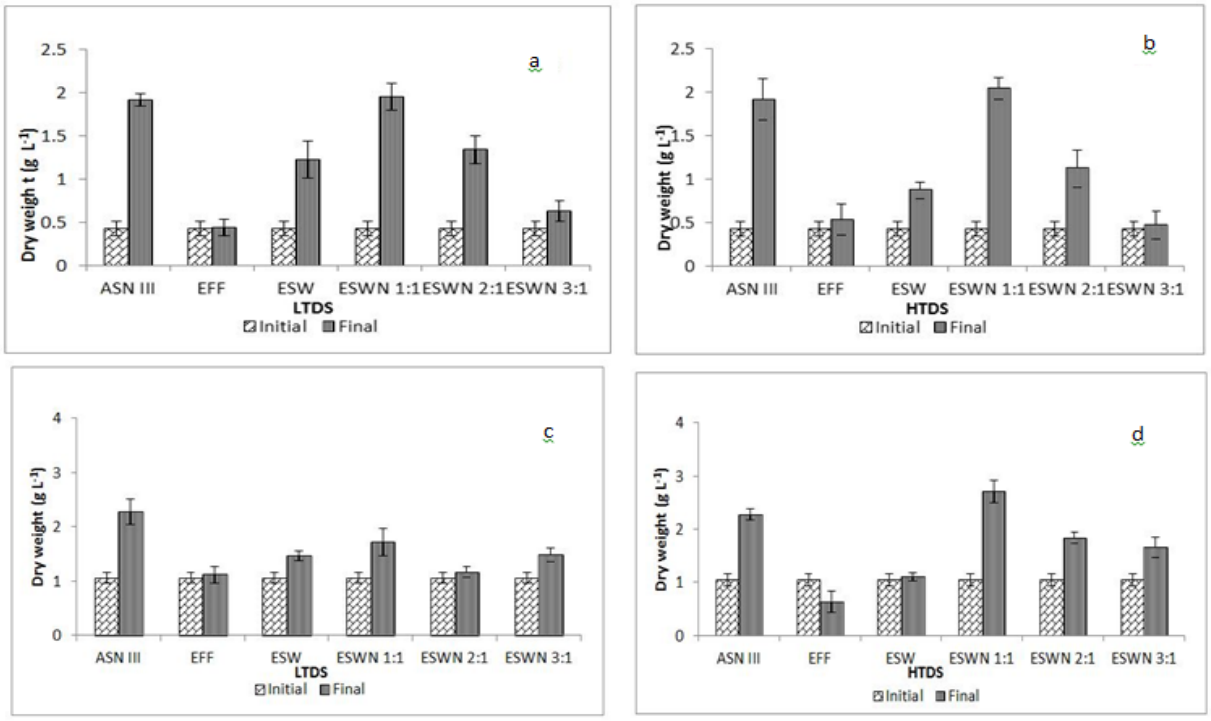

Figure 1: End point growth of *Oscillatoria willei* BDU130791 (a and b) and *Phormidium valderianum* BDU20041 (c and d) in different effluent sea water dilutions.

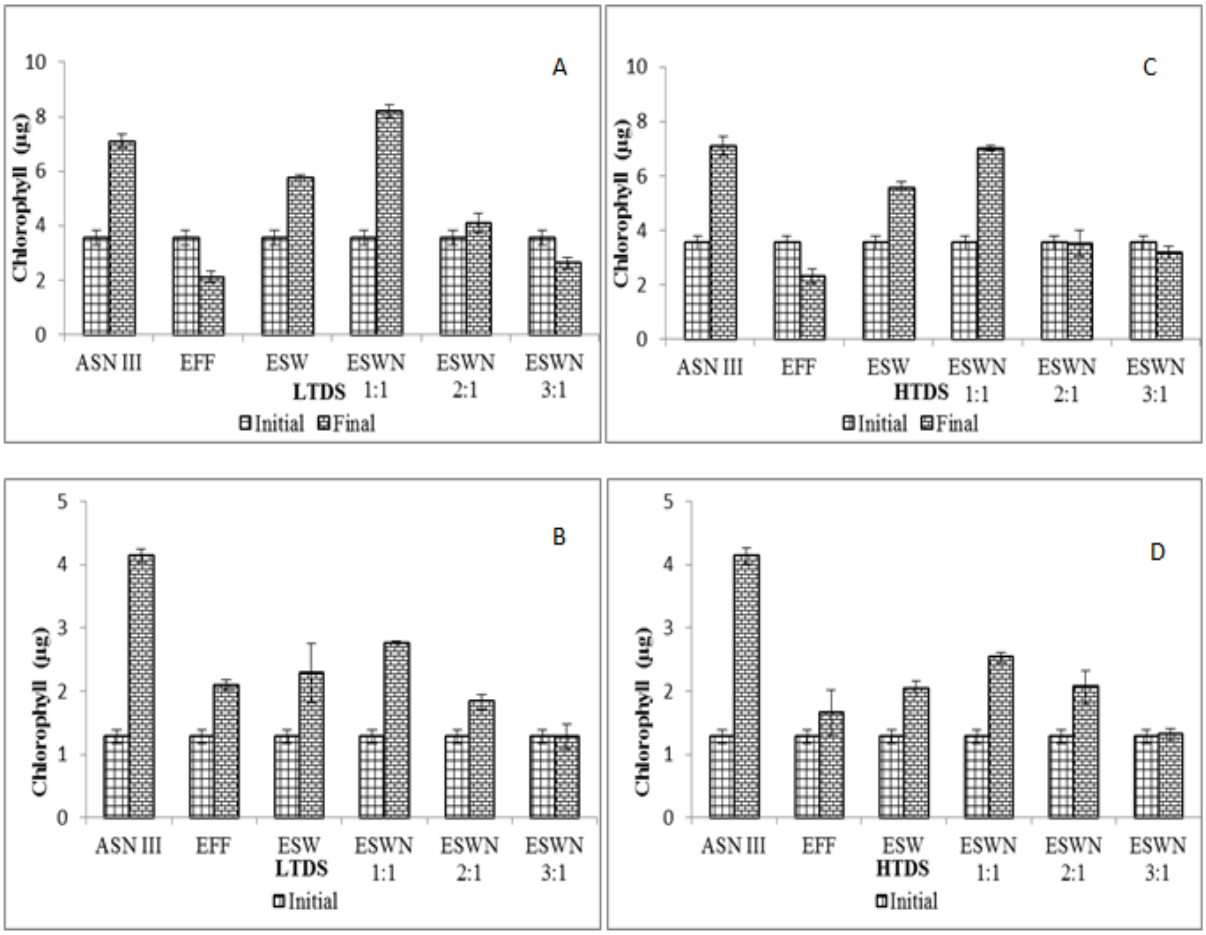

Figure 2: Chlorophyll concentrations of *Oscillatoria willei* BDU130791 (a and b) and *Phormidium valderianum* BDU20041 (c and d) in different effluent sea water dilutions.

standard deviation of the experimental results was calculated using MS-excel.

Results

Both the chosen strains when grown in three types of effluent, namely (DCP, HTDS and LTDS), the undiluted ossein effluent by itself supported trifling growth of the organism (Figure 1). In order to facilitate growth further the effluents were diluted with seawater in ratios of 1:1, 2:1 and 3:1; and amended with low-cost fertilizer grade nutrients -nitrogen (urea) and phosphorous (rock phosphate) sources.

Growth of marine cyanobacteria in ossein effluent

Of the three chosen effluents, both the marine cyanobacteria did not establish growth in di-calcium phosphate rich (DCP) effluent effectively in any of the dilution with seawater (data not shown) and was not further evaluated. Experiments were restricted to both HTDS and LTDS effluent, where opulent growth of the both strains was observed. From Figures 1 and 2 it is evident that in terms of biomass and chlorophyll a, *O. willei* BDU130791 could grow well in LTDS 1:1, Wherein *P. valderianum* BDU*20041* showed appreciable growth in HTDS 1:1 effluent. The dry weight of *O. willei* BDU*130791* as biomass component in diluted 1:1 LTDS effluent was 1.95 G L^{-1}, (Figure 1a) a fourfold increase over the raw effluent (0.44 G L^{-1}) and the initial inoculum (0.424 G L^{-1}). When grown in LTDS 1:1 effluent *O. willei* BDU130791 yielded a maximum of 8.21 µg chlorophyll *a* which was twice the initial inoculum (Figure 2a). The other selected strain *P. valderianum* BDU*20041* grown in HTDS 1:1 effluent showed a biomass yield of 2.712 G L^{-1} and 2.53 µg of chlorophyll as end point growth, which was three fold higher than the initial biomass (0.632 g L^{-1}) (Figures 1d and 2d) and twice than that of the initial chlorophyll (1.05 µg). The specific growth rate of both *O. willei* BDU130791 and *P. valderianum* BDU20041 was found to be 0.161 µ d^{-1} In LTDS 1:1 and HTDS 1:1 effluent respectively. Biomass productivity of 0.15 g L^{-1} d^{-1} By *O. Willei* BDU130791 in LTDS 1:1 and 0.06 G L^{-1} D^{-1} by effluent was presented in Table 1. From The Results, It Could Be Inferred That, *O. Willei* BDU130791 Could Grow Well In LTDS 1:1 Effluent and *P. Valderianum* BDU20041 In HTDS 1:1 and Further Studies Were Carried Out In The Respective Effluent.

Parametric analysis of Ossein effluent

The prime objective of any biological waste water treatment is to reduce the biological oxygen demand (bod), nutrient utilization (nitrate, nitrite, ammonia, phosphorous) and removal of suspended solids thus reducing the pollution load. The initial dissolved oxygen in LTDS 1:1 and HTDS 1:1 ossein effluent was 3.587 mg L^{-1} and 2.484 mg L^{-1} respectively. upon treatment with *O. willei* BDU130791 and *P.*

Sl. No	Organism	Effluent	Biomass productivity	Specific growth rate
1	*Oscillatoria willei* BDU130791	ESWN LTDS 1:1	0.15 g L^{-1} d^{-1}	0.161 μ d^{-1}
2	*Phormidium valderianum* BDU20041	ESWN HTDS 1:1	0.06 g L^{-1} d^{-1}	0.161 μ d^{-1}

Table 1: Biomass productivity and specific growth rate of *Oscillatoria willei* BDU130791 and *Phormidium valderianum* BDU20041 grown in half strength LTDS and HTDS effluent respectively.

No	Parameters		LTDS (mg L^{-1})	HTDS (mg L^{-1})
1	Ammonia (free ammonia)	Untreated	12.39 ± 0.18	11.25 ± 0.08
		Treated	8.63 ± 0.11	6.25 ± 0.05
2	Dissolved Oxygen	Untreated	3.58 ± 0.12	2.48 ± 0.04
		Treated	6.1 ± 0.02	5.81 ± 0.20
3	Total Kjeldahl Nitrogen	Untreated	184 ± 3.07	158 ± 2. 64
		Treated	91 ± 2.85	79 ± 2.44
4	Nitrate	Untreated	22.01 ± 0.03	26.04 ± 0.07
		Treated	4.41 ± 0.02	10.58 ± 0.05
5	Nitrite	Untreated	79.52 ± 1.12	79.22 ± 2.45
		Treated	71.41 ± 1.05	71.18 ± 0.04
6	Total phosphorous	Untreated	18.85 ± 0.23	23.45 ± 0.24
		Treated	9.49 ± 0.07	13.05 ± 0.03
7	Inorganic phosphorous	Untreated	62 ± 1.31	223 ± 7.17
		Treated	40.26 ± 1.03	180 ± 6.82
8	Calcium	Untreated	160 ± 5.23	1520 ± 14.3
		Treated	94 ± 1.67	1250 ± 12.2
9	Magnesium	Untreated	192 ± 3.56	312 ± 4.98
		Treated	125 ± 5.2	72 ± 0.13

Table 2: Summary of the results obtained in bioremediation of the ossein effluent by *Oscillatoria willei* BDU130791 and *Phormidium valderianum* BDU20041.

valderianum BDU20041 The do levels at the end of 7th Day Were 6.189 and 5.81 mg L^{-1} respectively (Table 2).

Total kjeldahl Nitrogen (TKN), sum of organic nitrogen, ammonia and ammonium showed significant reduction rates of 58% and 50% In LTDS 1:1 and HTDS 1:1 effluent by *O. willei* BDU130791 and *P. Valderianum* BDU20041 respectively. The initial nitrate concentrations Of 22.01 mg L^{-1} and 26.04 mg L^{-1} In The LTDS and HTDS effluent were reduced to 4.415 mg L^{-1} By *O. Willei* BDU130791 and 10.58 mg L^{-1} By *P. Valderianum* BDU20041 Implying The Growth Of The Organism (Table 2) The initial nitrite concentrations of the two effluents were 79.52 mg L^{-1} and 79.22 mg L^{-1} and the treated effluent recorded values of 71.41 and 71.18 mg L^{-1} (Table 2). The final mean concentration of the ammonia in the cyanobacterial treated ossein effluent LTDS and HTDS were 8.63mg L^{-1} and 6.25 Mg L^{-1} (Table 2) with a substantial reduction of 43% and 44% By *O. willei* BDU130791 and *P. valderianum* BDU20041 respectively.

In the present study *O. willei* BDU130791 removed 49.64% of total phosphorous and 35.06% of inorganic phosphorous in the LTDS effluent and HTDS effluent upon treatment with *P. valderianum* BDU20041 showed significant removal of 44.34 and 19.28% respectively.

Ossein effluent has high amount of calcium as calcium chloride, leached out during decalcification of the cattle bones. Upon treatment by marine cyanobacteria, *O. willei* BDU130791 significantly removed 41.25% of calcium and 34.8% of magnesium in LTDS effluent wherein, *P. valderianum* BDU20041 showed pronounced removal rates of 17.76% of calcium and 76.92% of magnesium in HTDS effluent (Table 2).

Characterization of marine cyanobacterial biocalcite from ossein effluent

Fourier transform infrared spectroscopy: Significant calcium removal rates by marine cyanobacteria necessitated the evaluation of the calcification potential and structural elucidation of the calcite formed.

Dried biomass of O. willei BDU130791 showed Bands at 1451.17, characteristic of a C-O stretch in the carbonate Ion [16] and the peak at 855.83 cm_{-1} is typical for the presence of calcium carbonate.

The precipitates on the growth vessel of both the organisms revealed a characteristic peak for calcite at 713.41 cm^{-1} and 712.24 cm^{-1}. The dry biomass *of O. willei* BDU*20041* grown in LTDS effluent showed peaks of calcium carbonate at 1451.17 and 855.83 cm^{-1} *while in P. valderianum* BDU20041 significant peaks of calcite at 1421.03 and 875.14 confirmed bio-mineralization potentials of organism in HTDS effluent. The band at 712 .24 cm^{-1} results from in-plane deformation vibrations of the planar CO_3 units and the band at 875.14 cm^{-1} are distinguished for the out-of-plane deformation vibrations of the planer CO_3 units respectively. Interestingly, as expected, the Asn III grown biomass of *O. willei* BDU130791 and *P. valderianum* BDU20041 didn't show any characteristic peak of calcite in its infrared spectrum.

Scanning electron microscopy and energy dispersive spectroscopy: Cyanobacteria the primordial photosynthetic organisms, exhibiting versatile physiology, and extended ecological survivability has been well established to precipitate carbonaceous substances both atmospheric and industrial source. The dried biomass and the precipitates were characterized by scanning electron microscopy and energy dispersive spectroscopy (EDS).

Interestingly *P. valderianum* BDU20041 revealed the bio-calcite to have completely layered the filaments (Figure 3c) while calcite crystals were found dispersedly adhered to the filaments of *O. willei* BDU130791 (Figure 3d). The calcium carbonate precipitates showed structures composed of irregularly distributed rhombhohedral and hexagonal crystals (Figure 3e and 3f). Similar structures were not observed in the

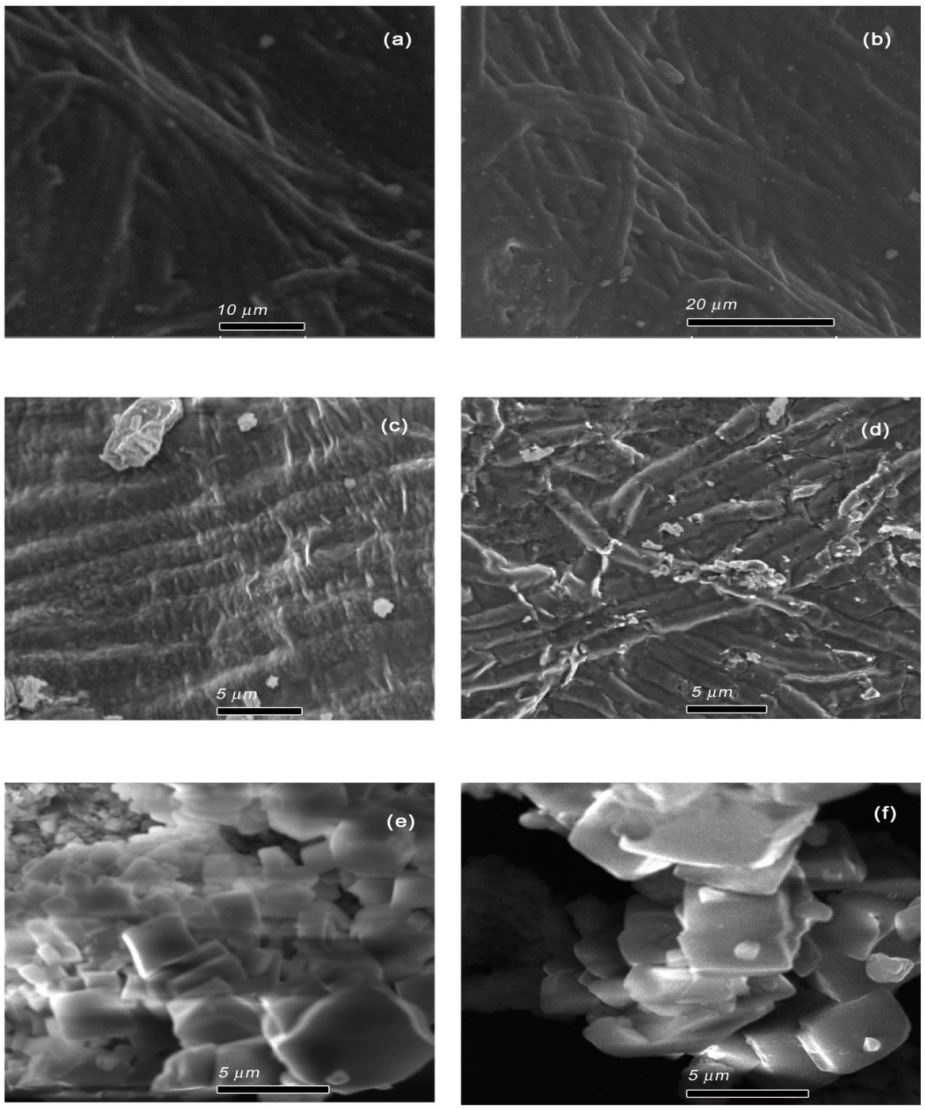

Figure 3: Scanning electron microscopic (SEM) images of *P. valderianum* BDU20041 and *O. willei* BDU130791. The filaments of *P. valderianum* BDU20041 (a) and *O. willei* BDU130791 (b) grown in ASN III medium. (c and d) reveal the calcium carbonate deposits as the calcite crystals over the filaments of *P. valderianum* BDU20041 and *O. willei* BDU130791. The precipitates formed during growth of *P. valderianum* (e) and *O. willei* (f) showed presence of rhambohedral structures typical of calcite.

ASN III grown strains which commemorates that bio mineralization by cyanobacteria has occurred in a calcium rich environment (Figure 3a and 3b).

The elemental composition of the biocalcite of the biomass and the crystals formed were analyzed by energy dispersive spectroscopy coupled to SEM. The spectrum of the biomass grown in ASN III medium (Figure 4a and 4b) revealed the presence of elements characteristic of the growth medium and no traces of calcium was recorded, wherein the eds spectrum of the biomass grown in ossein effluent showed peaks of C, Ca, Cl, Na, Mg, O and S of which oxygen, carbon, magnesium and calcium were the most prominent which imputes to the calcium and magnesium calcites (Figure 4c and 4d). EDS analyses of the calcareous particles from the HTDS and LTDS effluent show Mg-calcite as the dominating calcium carbonate polymorph affirming the bio mineralization by marine cyanobacteria (Figure 4e and 4f). The calcite's IR spectrum, morphological observation via SEM and the elemental analysis by EDS spectra ratifies the calcifying ability of the marine cyanobacteria and thus adds a new dimension to its widely reported bioremediation potentials.

Discussion

In the process of manufacturing gelatin, ossein the decalcified bone is the raw material effluent with its high calcium 30,000 mg L^{-1} and chloride 18,000 mg L^{-1} emerges is of major concern in its disposal.

In the process of manufacturing gelatin, ossein the decalcified bone with high calcium 30,000 mg L^{-1} and chloride 18,000 mg L^{-1} emerges and its disposal is of major concern. The calcium richness of the ossein effluent can be efficiently converted to calcium carbonate only by the biological process. In modern days, microalgal bioremediation has enticed prime attention owing to their pivotal role in CO_2 fixation, and its biomass utilization [19]. Biological remediation of waste water using indigenous microalgae is a sustainable approach [20] and

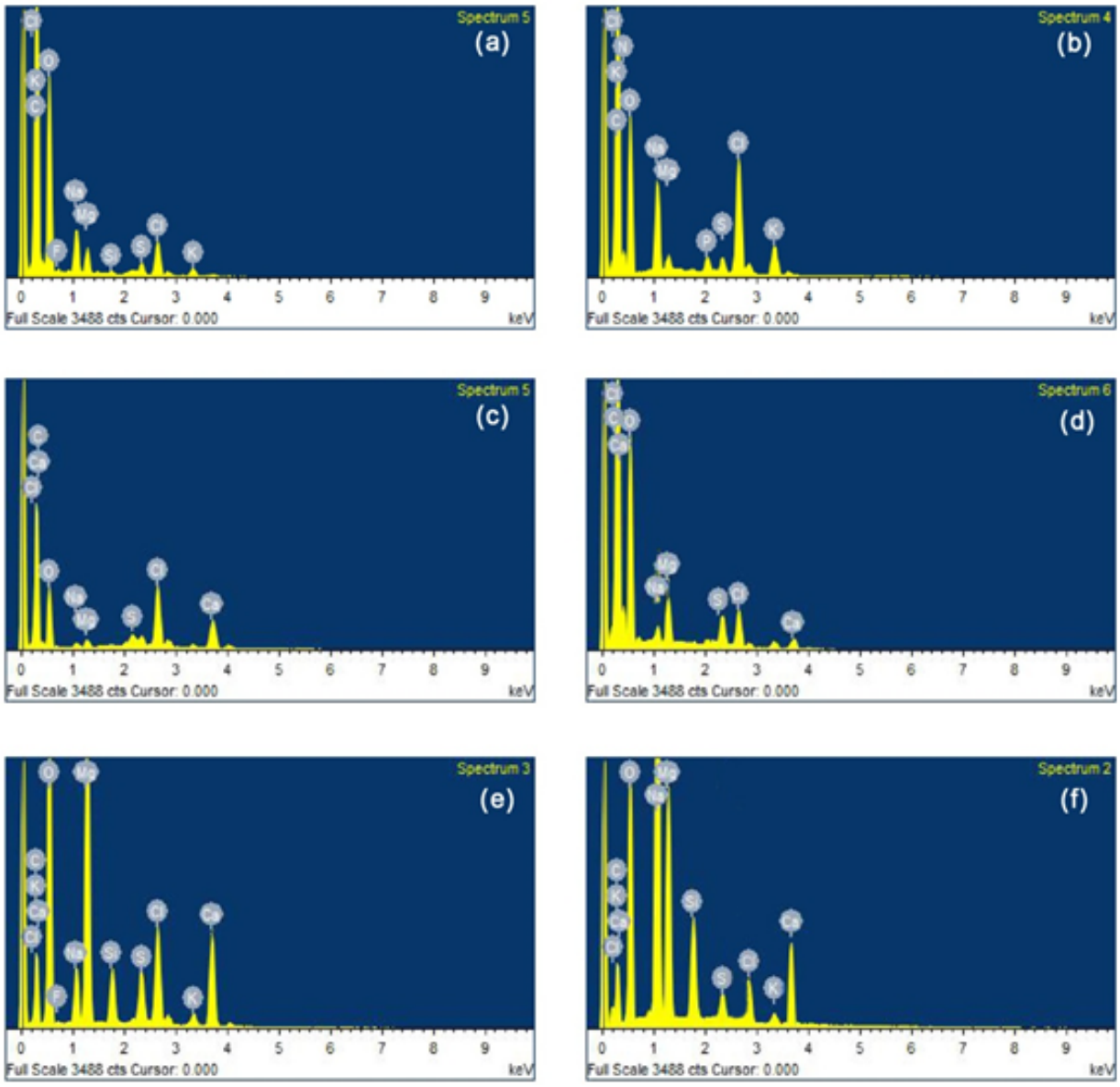

Figure 4: EDS spectra of the biomass of (a) *P. valderianum* BDU20041 and (b) *O. willei* BDU130791 grown in ASN III medium and in ossein effluent (c and d). Precipitates obtained by the growth of *P. valderianum* BDU20041 (e) and *O. willei* BDU130791 (f) in ossein effluent.

offers advantages such as nutrient recovery, biomass production and heavy metal adsorption [21]. Cyanobacteria the primordial oxygenic photosynthetic prokaryote surviving in a variety of environmental regimes [22] are efficient degraders of hard and hazardous substances. Arguably they are most attractive alternatives for remediation of calcium rich effluent as they are capable of sequestering calcium as insoluble $CaCO_3$ [9].

Growth of marine cyanobacteria in ossein effluent

Selection of an appropriate cyanobacterial species is critical in any bioremediation process, as the selected strain should provide high nutrient removal rates and appreciable biomass in the chosen effluent [23]. Inducing growth of the organism in wastewaters is vital in bioremediation as it will determine the efficacy and sustainability of the treatment. In the present study, the calcification potentials of two marine cyanobacteria *O. willei* BDU130791 and *P. valderianum* BDU20041 was evaluated. The strains were grown in three different ossein effluent DCP-di-calcium phosphate rich, HTDS-high total dissolved solids, LTDS-low total dissolved solids. The effluents varied in their amounts of total dissolved calcium and magnesium. The initial growth study was carried out to access the ability of the organism to adapt and grow in the effluent in different dilutions with seawater (raw effluent, 1:1, 2:1 and 3:1). Of the various dilutions tested, the half strength low TDS and high TDS effluent with added cost effective nutrients proved to be a suitable medium for marine cyanobacterial growth (Figures 1 and 2) of the two organisms *O. willei* BDU130791 showed appreciable growth in LTDS effluent 1:1 with a biomass productivity of 0.15 g d^{-1} and *P. valderianum* BDU20041 revealed a productivity of 0.06 g d^{-1} in HTDS 1:1 effluent (Table 1). It is well documented by the authors that marine cyanobacteria can grow and bioremediate varied type of effluents including ossein [6,24].

In the present study, the obtained results affirmed that the marine cyanobacteria *O. willei* BDU130791 and *P. valderianum* BDU20041 could be grown in LTDS and HTDS effluent 1:1 dilution with seawater. This further necessitates the evaluation of nutrient removal rates, biological oxygen demand (bod) and calcifying potentials towards the remediation of the ossein effluent. Of the two tested organisms, *O. willei* grown in LTDS effluent appreciably increased the DO levels by 72.53% over the initial do (3.58 mg l^{-1}) at the end of 7 days, wherein *P. valderianum* BDU20041 almost doubled the dissolved oxygen rates in HTDS effluent. Increased DO in the treated effluent is a valuable property as it will support and enhance the aquatic life [25], and would reduce the abnoxious odour.

Nutrient removal (nitrate and phosphorous) is another critical parameter in bioremediation and is attributed to the photosynthetic activity and biomass production [21]. Primary mechanism of total nitrate and phosphate assimilations are presumed to be biomass uptake, thus a decrease in the N and P details the exponential growth of organism in the effluent [26]. When grown in LTDS 1:1 effluent O. willei BDU130791 reduced Total Nitrate from 22.01 mg L^{-1} to 4.41mg L^{-1} and The levels in P. valderianum BDU20041 got reduced from 26.04 mg l^{-1} to 10.08 mg l^{-1} at the end of seventh day and the concurrent nitrite removal rates were about 11% in both the effluents. In the present study, the selected strains removed nearly 50% of total phosphorous from the respective ossein effluent. These results substantially evince the growth of the marine cyanobacteria in the ossein effluent. A total or near total removal of all forms of phosphate has been reported in *Oscillatoria Sp.* and *Aphanocapsa Sp.* from distillery effluent [27,28].

Calcification by Marine cyanobacteria

The total hardness of any waste water is attributed to its calcium and magnesium concentration. Carbon concentrating mechanism (CCM) exhibited by cyanobacteria is a metabolic system that enables the enrichment of co_2 at the sight of rubisco and calcification is a non-obligate and an integral process of cyanobacterial ccm. [29]. Ossein effluent contains rich amounts of calcium as calcium chloride or di calcium phosphate leached out during decalcification of the cattle bones. Significant reduction rates of 41.25% and 17.76% of calcium from LTDS and HTDS ossein effluent at the end of 7th day purports the utilization of marine cyanobacteria towards low cost treatment of ossein effluent by calcification.

Characterization of marine cyanobacterial bio calcite from ossein effluent

Fourier transform infrared spectroscopy: The IR spectrum of the dried biomass of both the strains revealed fundamental bands of calcite at 855.83 cm^{-1} in LTDS 1:1 effluent by *O. willei* BDU130791 and the one at 875.14 cm^{-1} in HTDS 1:1 effluent by *P. valderianum* BDU20041. This peak indicates the typical calcium carbonate peak as reported by Zhao et al. [17] and Nasrazadani et al. [18]. Calcite is a stable modification of $CaCO_3$ which forms a mat eventually owing to the nucleation of the crystals. Aragonite is less stable form than calcite and grows as unattached orthorhombic crystals and often transforms to calcite. Vaterite is the least stable form and it usually gets converted to either calcite or aragonite in course of time [30]. Interestingly, The distinct peaks at 875 and 855 cm^{-1} observed in effluent grown cultures was totally absent in the IR spectrum of the organisms grown in ASN III medium, proves evidently that, the induction of calcification is only by the two tested organisms *P. valderianum* BDU20041and *O. willei* BDU130791.

The other peaks at 1430, 875 and 712 cm^{-1}, are attributable to the vibration of carbon and oxygen double bond in the carbonate ion and the peak at 875 cm^{-1} can be more precise in identifying calcium carbonate by ir analysis [31]. Peak of the dried biomass of O. willei BDU130791 indicates the characteristic C-O stretching of the carbonate Ion [16]. The rest of the frequencies above 1000 cm^{-1} correspond to the symmetric and asymmetric stretching modes of carbonate [32]. The IR spectrum of the effluent grown organisms thus revealed the organisms' ability to precipitate calcium as calcium carbonate and is directly accredited to the reduction of hardness in the treated ossein effluent.

Scanning electron microscopy and energy dispersive spectroscopy: The structural elucidation of the calcite was further studied by scanning electron microscopy coupled with electron dispersive spectroscopy. Examination of the effluent grown dried biomass evidently revealed the complete coating of $CaCO_3$ precipitates on the filaments of *P. valderianum* BDU20041 (Figure 3c), wherein *O. willei* BDU130791 showed random deposits of calcite over the filaments (Figure 3d) called whitings. Calcification occurs as dense and most likely as unequal encrustation around the cyanobacterial sheath as calcareous tubes [33]. Biologically catalyzed calcites are called whitings and its occurance is closely associated with the cyanobacterial population and alkalinity changes in any habitat [34]. These results evidently ratify the bio mineralization potential of marine cyanobacteria. Further morphological observation of the deposits obtained from the side walls of the flasks reveal the presence of rhombohedral and rectangular shaped crystals of calcium carbonate asserting active biocalcification by the selected marine cyanobacteria grown in ossein Effluent (Figure 3e and 3f). Earlier Lee et al. [34] reported the calcification potential of unicellular Cyanobacteria *Synechocccus Sp.* Pcc8806 and *Synechocystis Sp.* Pcc8807. Thus, the present study has for the first time resulted in the utilization of filamentous non-heterocystous cyanobacteria towards biocalcification mediated bioremediation of ossein effluent. Mineralogical analysis of the dried biomass and the $caco_3$ precipitate substantiates our results with the presence of calcium and magnesium polymorphs in the treated biomass and the crystals which further purports the bio calcification by marine cyanobacteria. The calcium carbonate precipitate obtained in our study is of Mg-aragonite type and a similar type has also been reported to have formed by cyanobacteria in natural habitats of North Sea. Further the biomass obtained upon treatment of the calcium rich ossein effluent can be exploited biotechnologically. The wastewater treated algal biomass has been utilized for biodiesel and bioethanol production [35] in algal ponds and closed systems respectively. Thus cyanobacteria, the largest group of prokaryotes could be adjudged as the most pertinent organism in the cost effective treatment of ossein effluent and thus postulates a novel approach.

Conclusion

Calcium rich ossein effluent from a gelatin producing industry was treated by *O. willei* BDU130791 and *P. valderianum* BDU20041 by Its bio calcification potential. The findings indicate the potentials of marine cyanobacteria mediating $CaCO_3$ precipitation to bioremediate the calcium rich effluent. The results show calcification which appears to be a plausible alternative for calcium abatement in effluent and CO_2 sequestration from point source - one of the best biological remediation process which is cost effective.

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References

1. Subramanian G, Prabaharan D (1994) Cyanobacterial hydrogen production. Journal of Swamy Botanical Club 11: 1-11.
2. Shashirekha S, Uma L, Subramanian G (1997) Phenol degradation by a marine cyanobacterium *Phormidium valderianum*. Journal of Industrial Microbiology and Biotechnology 19: 130-133.
3. Swaminathan P, Krishnan K, Uma L (2012) Tannase activity from the marine cyanobacterium *Phormidium valderianum* BDU140441. Journal of Applied Phycology 24: 1093-1098.
4. Swaminathan P, Prabaharan D, Uma L (2009) Fate of few pesticide-metabolizing enzymes in the marine cyanobacterium *Phormidium valderianum* BDU20041 in perspective with chlorpyrifos exposure. Pesticide Biochemistry and Physiology 94: 68-72.
5. Priya B, Uma L, Ahamed AK, Subramanian G, Prabaharan D (2011) Ability to use the diazo dye, C.I.Acid Black 1 as a nitrogen source by the marine cyanobacterium *Oscillatoria curviceps* BDU 92191. Bioresource Technology 102: 7218-7223.
6. Kalavathi DF, Uma L, Subramanian G (2001) Degradation and metabolization of the pigment-melanoidin in distillery effluent by the marine cyanobacterium *Oscillatoria boryana* BDU 92181. Enzyme Microbial Technology 29: 246-251.
7. Rashmi V, ShylajaNaciyar M, Rajalakshmi R, D'Souza SF, Prabaharan D, et al. (2013) Siderophore mediated uranium sequestration by marine cyanobacterium *Synechococcus elongates* BDU 130911. Bioresource Technology 130: 204-210.
8. Ramanan R, Kannan K, Deshkar A, Yadav R, Chakrabarti T (2010) Enhanced algal CO_2 sequestration through calcite deposition by *Chlorella* sp. and *Spirulina platensis* in a mini-raceway pond. Bioresource Technology 101: 2616-2622.
9. Jansson C, Northen T (2010) Calcifying cyanobacteria-the potential of biomineralization for carbon capture and storage. Current Opinion Biotechnology 21: 365-371.
10. Lee BD, Apel WA, Walton MR (2004) Screening of cyanobacterial species for calcification. Biotechnology Progress 20: 1345-1351.
11. Rippka R, Deruelles J, Waterbury JB, Herdman M, Stainer RY (1979) Generic assignments, strain histories and properties of pure cultures of cyanobacteria. Journal of General Microbiology 111: 1-61.
12. Mac Kinney G (1941) Absorption of light by chlorophyll solutions. Journal of Biological Chemistry 140: 314-322.
13. Tang D, Han W, Li P, Miao X, Zhong J (2011) CO_2 biofixation and fatty acid composition of *Scenedesmus obliquus* and *Chlorella pyrenoidosa* in response to different CO_2 levels. Bioresource Technology 102: 3071-3076.
14. Levasseur M, Thompson PA, Harrison PJ (1993) Physiological acclimation of marine phytoplankton to different nitrogen sources. Journal of Phycology 29: 587-595.
15. APHA, AWWA, WEF (American Public Health Association, American Water Works Association. Water Environment Federation) (1999) Standard Methods for the Examination of Water and Wastewater 20th ed. Washington DC.
16. Legodi MA, de Wall D, Potgieter JH, Potgieter SS (2001) Rapid determination of $CaCO_3$ in mixtures utilising FT-IR spectroscopy. Miners Engineering 14: 1107-1111.
17. Zhao W, Yang H, Huo C (2012) Surface nanocrystallization modification of anhydrite. Colloids and Surfaces A: Physicochemical and Engineering Aspects 393: 128-132.
18. Nasrazadani S, Namduri H (2006) Study of Phase Transformation in Iron Oxides Using Laser Induced Breakdown Spectroscopy (LIBS), Spectrochimica Acta Part B 61565-61571.
19. Lim SL, Chu WL, Phang SM (2010) Use of *Chlorella vulgaris* for bioremediation of textile wastewater. Bioresource Technology 101: 7314-7322.
20. El-Bestawy EA, El-Salam AZ A, Mansy AERH (2007) Potential use of environmental cyanobacterial species in bioremediation of lindane-contaminated effluents. International Journal of Biodeterioration & Biodegradation 59: 180-192.
21. Riaño B, Molinuevo B, García-González MC (2011) Treatment of fish processing wastewater with microalgae-containing microbiota. Bioresource Technology 102, 10829-10833.
22. Saha SK, Uma L, Subramanian G (2003) Nitrogen stress induced changes in the marine cyanobacterium *Oscillatoria willei* BDU 130511. FEMS microbiology ecology 45: 263-272.
23. De Godos I, Guzman HO, Soto R, García-Encina PA, Becares E, et al. (2011) Coagulation/flocculation-based removal of algal–bacterial biomass from piggery wastewater treatment. Bioresource Technology 102: 923-927.
24. Chellapandi P, Prabaharan D, Uma L (2009) Evaluation of Methanogenic Activity of Biogas Plant Slurry for Monitoring Codigestion of Ossein Factory Wastes and Cyanobacterial Biomass. Applied Biochemistry and Biotechnology 162:524-535
25. Raouf NA, Al-Homaidan, AA, Ibraheem IBM (2012) Microalgae and wastewater treatment. Saudi Journal of Biological Science 19: 257-275.
26. Cho S, Luong TT, Lee D, Oh YK, Lee T (2011) Reuse of effluent water from a municipal wastewater treatment plant in microalgae cultivation for biofuel production. Bioresource Technology 102: 8639-8645.
27. Vijayakumar S (2005) Studies on cyanobacteria in industrial effluents-An environmental and molecular approach. PhD thesis submitted to Bharathidasan University, Tiruchirapalli, Tamil Nadu, India.
28. Boominathan M, Sundararaman M, Manoharan C (2007) Biodiversity of microbes in dairy effluent. Pollution Research 2: 271-276.
29. Kupriyanova EV, Sinetova MA, Cho SM, Park YI, Los DA, et al. (2013) CO_2-concentrating mechanism in cyanobacterial photosynthesis: organization, physiological role, and evolutionary origin. Photosynthetic Research 117: 133-146.
30. Plavsic B, Kobe S, Orel B (1999) Identification of crystallization forms of $CaCO_3$ with FTIR spectroscopy. Metals Alloy Technology 33: 517-521.
31. Matsuda Y, Tsukada M (1995) Identification of calcium carbonate contained as body in modern paints by FTIR spectroscopy. IRUG Postprints 25-34.
32. Tatzber M, Stemmer M, Spiegel H, Katzlberger C, Haberhauer G, et al. (2007) An alternative method to measure carbonate in soils by FT-IR spectroscopy. Environmental Chemistry Letters 5: 9-12.
33. Martina Merz-Preib, Robert Riding (1999) Cyanobacterial tufa calcification in two fresh water streams: ambient environment, chemical thresholds and biological processes. Sedimentary Geology 126: 103-124.
34. Lee K, Lee CG (2001) Effect of light/dark cycles on wastewater treatments by microalgae. Biotechnology and Bioprocess Engineering 6: 194-199.
35. Park JBK., Craggs RJ, Shilton AN (2011) Wastewater treatment high rate algal ponds for biofuel production. Bioresource Technology 102: 35-42.