ORIGINAL ARTICLE



Evaluation of self-purifying power of cyanobacteria *Pseudanabaena* galeata: case of dairy factory effluents

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Abstract

This study aims to evaluate the treatment of wastewater from dairy plants using the self-purifying power of filamentous cyanobacterium *Pseudanabaena galeata*. The performance and the reliability of using cyanobacteria as wastewater treatment technique were also assessed based on total bacterial flora, total nitrogen (NK), chemical oxygen demand (COD), and total organic carbon (TOC). The results obtained show promising treatment of wastewater if 25% and 50% dilutions were applied. Thus, respectively, for 25 and 50% dilutions, the reduction of the total bacterial flora was 67 and 45%, NK removal was close to 92 and 77%, the COD decreased by 77% and 65%, and the TOC decreased by 78 and 77%. The final concentration of cyanobacteria biomass increased from 15.72 to 585 mg/l and 475 mg/l, respectively, for 25 and 50% dilutions. These results suggest that cyanobacteria (*Pseudanabaena galeata*) may play an effective role in the biological treatment of wastewater.

Keywords Treatment · Pseudanabaena galeata · Self-purifying · Wastewater · Diary factory

Introduction

Water resources in Morocco are limited due to the semiarid climate that characterizes most of the country that often undergoes episodic droughts. This issue is not limited to the quantity of water resources, but also to their quality, which attracts global intention more than ever nowadays (EL Addouli et al. 2009). Increasing population and industrialization are key contributors to water pollution (Rawat et al. 2011; Sood et al. 2012). These wastewaters are highly hazardous for ecosystem and human health. This issue requires a comprehensive and effective treatment before discharge into water bodies (Mishra and Mishra 2017).

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In Morocco, domestic and industrial wastewaters are often reused without prior treatments, leading to major risk for public health such as epidemics including typhoid and cholera. The protection of public health and the environment is now the country's major concern (Darley 2002; Souabi 2007; Vasel and Jupsin 2007). Thus, wastewater treatment is becoming a priority today to reuse in agriculture, industry, and other sectors.

A number of physical, chemical, and biological methods have been developed for the treatment of wastewaters; among these, the use of microalgae is considered as an ecofriendly and low-cost approach. Biological processes for wastewater purification have shown a promising future as an alternative system for wastewater treatment (Pouliob and Notie 1985; Kong et al. 2010; Su et al. 2011).

Microalgae cultivation is an interesting approach for wastewater treatment. This approach provides tertiary biotreatment coupled with potentially interesting biomass production, which can be used for many purposes including bioenergy, pharmaceuticals, organic fertilizers, biofuels, and food for animals (Abdel-Raouf et al. 2012; Batista et al. 2015; Cai et al. 2013; Gouveia 2011; Phasey et al. 2017; Schenk et al. 2008; Renuka et al. 2015).

Cyanobacteria (green blue algae) are prokaryotic autotrophic organisms capable of surviving in adverse



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environments (wastewater). Their growth in polluted waters improves the quality of contaminated habitats. Cyanobacteria have high nitrogen and phosphorus nutrient requirements; thus, wastewaters can be used as growth media for cyanobacteria and indirectly reduce nutrient loading, making these waters useful for other applications. Apart from this, cyanobacteria have been shown to be effective in removing other contaminants such as heavy metals and inorganic and organic contaminants from various wastewaters (Sood et al. 2012). Cyanobacteria have the capacity to assimilate nutrients by phytoremediation, which refers to the dissipation of organic and inorganic compounds (carbon, nitrogen, or phosphorus), metals, and emerging contaminants from wastewater (Cuellar-Bermudez et al. 2016; Korosh et al. 2018). As a result, the integration of cyanobacteria into the wastewater treatment process seems a promising method since the removal of nitrogen and phosphorus is a key part to produce an effluent of an acceptable quality for irrigation (Korosh et al. 2018; Liu and Vyverman 2015; Sheekh et al. 2014).

Cyanobacteria are considered promising platforms to produce high-value fuels and/or chemicals in "bio-refineries". The treatment of wastewaters by cyanobacteria is an ecological approach and a process without secondary pollution as long as the biomass produced is reused and allows efficient recycling of nutrients (Munoz and Guieysse 2008). Cyanobacteria are common in eutrophic natural waters. Favoured by warm, stable, and nutrient-enriched waters, they can be an important part of the phytoplankton community in wastewater treatment ponds. There are many benefits of using photosynthetic organisms to improve the quality of wastewaters. The oxygen produced by photosynthesis is used to reduce biological oxygen demand and refractory organic compounds (Oswald et al. 1978). The high pH values, typical of photosynthetic organism cultures, cause the depletion of coliforms and other enteric bacteria (Marais 1974). However, many cyanobacteria like Pseudanabaena galeata produce toxic compounds, presumably for self-defence. Theses cyanotoxins can affect aquatic and terrestrial wildlife and humans (Oudra et al. 2002).

However, several procedures have been developed to monitor toxins usually stored inside the cells. These procedures include sedimentation, coagulation/flocculation processes, ozonation, and filtration technologies (Miao and Tao 2009; Miao et al. 2010; Roegner et al. 2014; Romero et al. 2014).

This work aims to study the self-purifying power of cyanobacteria species (*Pseudanabaena galeata*), isolated from the water dams near Tetouan city (Northern Morocco). This study allows us to monitor the behaviour of this species versus the pollutant load of dairy industrial effluents and to evaluate the quality of the effluent according the national norms of industrial liquid discharge.



Material and methods

Study site and sampling

The overall objective of this study is to evaluate the behaviour and the growth mode of cyanobacteria subjected to different concentrations of wastewater of dairy liquid discharge (COLAINORD Dairy Cooperative), which produces yogurt and various dairy products.

In addition to the polluting bacterial load, this wastewater contains dairy by-products (Table 1) usually evacuated to Oued El Maleh, located north of Tetouan city (Stitou 2008). Samples were collected inside the plant before any treatment. Samples analyses were done a few hours later at the laboratory of Faculty of Sciences-Tetouan (Table 1).

Biological material

The cyanobacterium *Pseudanabaena galeata* used in this study (Fig. 1) was isolated from the Nakhla Dam (south of Tetouan city) and then purified in a series of axenic cultures in BG13 medium (Ferris and Hirsch (1991). The species was identified according to the work of Komárek and Anagnostidis (2005), Acinas et al. (2009) and based on its fatty acid composition (Ouhsassi et al. 2017), which is generally used as a phylogenetic marker to characterize different species of cyanobacteria (Los and Mironov 2015).

The experiments were carried out based on a series of dilutions (25%, 50%, and 75%) made from the raw wastewater sample mixed with *Pseudanabaena galeata* inoculum (ww25% + Pseud; ww50% + Pseud; and ww75% + Pseud). In addition, BG130 which is a BG13 nitrogen-free and raw wastewater (ww control), both mixed with *Pseudanabaena*, and both were used as reference media (BG130 + Pseud; ww100% + Pseud) (Figs. 2, 3).

The *Pseudanabaena galeata* was previously subjected to a BG130, a free nutrient medium, for complete depletion of cell reserves prior to inoculation into wastewater samples.

 Table 1 Raw contents of sampled wastewater from dairy industrial plant effluents

Parameters	Values Mini	Values Max
Dry residue mg/l	1000	3000
Albumin concentration mg/l	700	1800
Grease mg/l	300	400
Lactose mg/l	300	900
Oxidability mg/l	1000	4000







Fig. 3 Appearance of wastewater samples at the end of treatment

Method used

The study will focus on the evaluation of the following parameters:

- The monitoring of temporal evolution of the biomass produced by the cyanobacterium *Pseudanabaena galeata* (Fig. 4)
- Total bacterial flora (Figs. 5, 6)
- Total dissolved nitrogen (Kjeldahl nitrogen) (Fig. 7)
- Chemical oxygen demand (COD) (Fig. 8) and
- Total organic carbon (TOC) (Fig. 9).

To do this, 5% of *Pseudanabaena* suspension consisting of some colonies is resuspended into 6 Erlenmeyer of 600 ml of wastewater each. The incubations were carried out in a thermostatically controlled culture chamber at 25 ± 1 °C, aerated with a continuous supply of air, and illuminated by fluorescent white lamps with a 24-h photoperiod 16-h day/8-h night.

Pseudanabaena galeata biomass was evaluated by the dosage of chlorophyll "a" following the method of Marker et al. (1980).



Fig. 2 Appearance of wastewater samples at the start of treatment with cyanobacteria Pseudanabaena galeata



Fig. 6 Behaviour of the total bacterial flora exponential growth in the presence and absence of cyanobacteria Pseudanabaena galeata on the same samples of experiment (wastewater control, ww25% + Pseud, ww75% + Pseud, and ww + Pseud)

The temporal evolution of heterotrophic bacteria was enumerated, by indirect count of CFU, on nutrient (PCA) incubated at 37.5 °C for 24 h. The rate of cell growth is determined by the formula: $\mu = \left(\left(\log_{10} N - \log_{10} N_0 \right) 2.303 \right) / \left(t - t_0 \right),$

where N = number of cells at time t and N_0 the number of initial cells.



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The nitrogenous matter is determined according to Kjeldahl method. This reference method is based on the transformation of organic nitrogen into inorganic nitrogen in the form of $(NH_4)_2SO_4$ by the oxidative action of sulfuric acid boiling on the organic matter and in the presence of a mineralisation catalyst. The procedure of Havilah et al. (1977), which facilitates the rapid analysis of many series of samples, is applied to products of digestion obtained from similar model as Kjeldahl method.

INCUBATION D⁻¹

The COD is the amount of oxygen required for the degradation of organic matter chemically. The wastewater sample is oxidized by a hot sulfuric solution of dichromate of potassium with silver sulphate as a catalyst. Chlorides are masked by mercury sulphate. The concentration



of green Cr^{3+} ions is then dosed by photometry (Rodier 2009,1996).

The total organic carbon (TOC) assessment is based on the chemical oxygen demand according to the COD/OC ratio estimated for the bacteria, algae, organic matter released by the algae, and wastewater (Akiyama 1973; Somiya and Fujii 1984).

The data of the initial measurements of the parameters recorded on the wastewater at the beginning of the tests are prescribed in Table 2. Note that the initial *Pseudanabaena* inoculum of chlorophyll "a" concentration is 15.72 mg/ml.

Result and discussions

Growth of cyanobacteria

After sampling from the Dam of Nakhla, 10 km south of Tetouan, the Pseudanabaena was purified after several treatments with BG13. The reduction of contaminating bacteria is achieved by the removal of citric acid from BG13 medium, the only source of organic carbon in this medium (Ferris and Hirsch 1991). The results obtained after 13 days of growth show an exponential growth rate of the cyanobacterial strain in most of the tested culture media with a notable growth in the case of (ww25% + Pseud). The exponential growth rate of Pseudanabaena galeata was significantly higher (p < 0.05) (0.30 d⁻¹) compared to other samples (0.28 d^{-1}) , (0.17d^{-1}) , and (0.12 d^{-1}) , respectively, for (ww50% + Pseud), (ww75% + Pseud), and (ww100% + Pseud). For the sample (ww25% + Pseud), the chlorophyll "a" increased from 15.72 to 585 mg/l. In the case of sample (ww50% + Pseud), the chlorophyll "a" increased from 15.72 to 475 mg/l. The cell growth of Pseudanabaena under the lack of nitrogen (NK) appears to be limited (less than 40 mg/l) in the control medium (BG130) (Fig. 4).

The high level of chlorophyll "a" was observed for the samples (ww25% + Pseud) (585 mg /l) and (ww50% + Pseud) (475 mg /l). The low level of chlorophyll "a" was observed in the samples (ww75% + Pseud) (123.55 mg/L) and (ww100% + Pseud) (64.46 mg/L). These results are in agreement with several works and studies. In fact, cells of filamentous cyanobacterium *Pseudanabaena* galeata can store excess quantities of nutrients, but can grow in low quantities of nutrients (Markou et al. 2014) and use their reserves in nitrogen to survive. This is consistent with the experiments of Sabour et al. (2009) who showed, as with phosphorus experiments, that cell growth of M. ichthyoblabe was substantially favoured under high nitrate concentrations, whereas cell growth under N-free or N-deficient conditions tends to be limited. It seems that Microcystis cells use their reserves in nitrogen to survive such conditions. The internal nitrogen allocations allow four days of growth for Microcystis and Synechocystis on nitrogen-deficient media. Pseudanabaena Sp. and Oscillatotia Sp. were grown in nitrogen-deficient media. The results showed that Phycobilin cells content decreased sharply for both strains (Loura et al. 1987). Nitrogen is the second most abundant element in microalgal biomass, and its content ranges from 1 up to 14% (typically around 5-10%) of dry weight (Grobbelaar 2004).

Daniel and Aubert (1968) observed a higher growth of phytoplankton in wastewater than that obtained with the optimal artificial media. A proposed hypothesis suggested that heterotrophic bacteria produce enough growth factors/regulators for cyanobacteria to thrive in wastewater. Heterotrophic bacteria play a ubiquitous role in the growth and survival of algae (Amin et al. 2015; Gonzalez and Bashan 2000; Kim et al. 2014; Seyedsayamdost et al. 2011). They open the possibility of revisiting the global carbon cycle and other biogeochemical processes (Amin et al. 2012; Landa et al. 2015). They can activate algal growth by producing carbon dioxide (Tison and Lingg 1979; Chirac et al. 1985), vitamins, and other growth factors/regulators (Stewart and Daft 1977a, b; Hino 1984). Escher and Characklis (1982) reported that, in several aquatic environments and considering the CO₂/O₂ ratio, photosynthetic carbon fixation is limited in the aqueous phase. Microalgae such as cyanobacteria produce a large amount of oxygen (0_2) by photosynthesis, the accumulation of which inhibits carbon fixation and consequently reduces the growth of algae (Coleman and Colman 1980). However, the reduction by heterotrophic bacteria of the tension exerted by dissolved oxygen can also be taken into account for the interpretation of the increase in algae growth. Heterotrophic bacteria degrade organic matter,

Table 2 Di	stribution of the
initial conc	entrations of the
different w	astewater samples
(ww) at the	e start of the analyses





consume oxygen, and produce CO2 and nutrients (nitrogen and phosphorus). Lange (1973) and Wang and Priscu (1994) showed that the growth of cyanobacteria was enhanced in the presence of bacteria. Fuentes et al. (2016) suggested that massive algae production can occur in the presence of bacteria, and microalgae–bacteria interactions can be beneficial to mass production of microalgae and algal products.

Removal of total bacterial flora

The growth of heterotrophic bacteria has been affected by the presence of *Pseudanabaena galeata* and is progressively decreasing from 75 to 6.5×10^4 CFU/ml (- 91.33%), from 150 to 39×10^4 CFU/ml (- 74%), from 225 to 49.3×10^4 CFU/ml (- 78.08%), and from 300 to 196×10^4 CFU /ml (- 34.66%), respectively, for (ww25% + Pseud), (ww50% + Pseud), (ww75% + Pseud), and (ww100% + Pseud). However, wastewater samples without *Pseudanabaena* increased from 300 to 540×10^4 cfu /ml (+ 80%) (Figs. 5, 6).

It seems that cyanobacterial secondary metabolites may act as antibacterial compounds leading to the disruption of bacterial communities and thus increases the efficiency of self-purification processes. Studies reveal that the antibacterial potential differs among cyanobacterial species, but appears to be more effective on Gram (+) bacteria (Martins et al. 2008). Moawad (1968) found that environmental factors favourable to algal growth were unfavourable to coliform survival (Oufdou et al. 2000). Cyanobacterial exudates can stimulate or inhibit other members of the microbial community, including heterotrophic bacteria (Cole 1982; Ostensvik et al. 1998). The antimicrobial compounds found in cyanobacterial exudates include polyphenols, fatty acids, glycolipids, terpenoids, alkaloids, and a variety of bacteriocins yet to be described (Borowitzka 1995). Bacterial disintegration could be attributed to the self-purification processes carried out by Pseudanabaena galeata. The increase in pH associated with photosynthetic processes leads to an increase in the coliform mortality rate (El Hamouri et al. 1995; Granum and Myklestad 2002). Algae significantly reduce E. coli contamination in eutrophic lakes by increasing oxygenation and pH (Ansa et al. 2011). On the other hand, Jupsin et al. (2004) stated that the combined effect of light, pH, and temperature improves the elimination of bacteria. In addition, nutrient competition in discontinuous environments can reduce the growth rate of bacteria. Nevertheless, our results were in agreement with the work of (Lange 1973), who showed that cultures of various cyanobacteria were improved in the presence of bacteria.

Removal of total nitrogen

The experimental results show that the total nitrogen concentrations decreased progressively and proportionally with the production of algal biomass. In fact, it decreased from 14.75 to 2 mg N /l (- 86.44%), 29.5 to 10 mg N/l (- 66.1%), 44.25 to 28 mg N/l (-36.72%), and 59 to 37 mg N/l (-37.28%), respectively, for (ww25% + Pseud), (ww50% + Pseud), (ww75% + Pseud), and (ww100% + Pseud) (Fig. 7). Liu and Vyverman (2015) indicated that Pseudanabaena sp. was highly suitable for removing nitrogen from wastewaters with high N/P ratio. The Pseudanabaena galeata strain uses the nitrogen to satisfy their needs for growth and survival. de la Noue and Basseres (1989) and Rasoul-Amini et al. (2014) suggested that the nearly total depletion of total nitrogen in a domestic wastewater culture medium was mainly attributed to algal consumption. Meanwhile, the degradation of organic compounds containing nitrogen by microalgae-bacteria consortia was significantly higher, and the nitrogen released was assimilated by the microalgae (Subashchandrabose et al. 2011). In this study, the best yield is observed for the case of (ww25% + Pseud) sample which recorded an assimilation of the dissolved nitrogen of 14.75 to 2 mg N/ml (- 86.44%).

Chemical oxygen demand reduction

Chemical oxygen demand (COD) is one of the parameters for measuring the quality of wastewater. It represents the amount of oxygen needed to oxidize all the organic matter in the water. The effect of *Pseudanabaena galeata* on COD reduction was carried out 13 days continuously. Figure 8 shows that the degradation process of the organic matter is expressed by a progressive decrease in the COD as a function of time for all the samples of experiment. Respectively, this recorded decrease was 37.5–10 mgO2/l (-73.33%); 75–32.5 mgO2/l (-56.66%); 112.5–78.5 mgO2/l (-30.22%); 150–92 mgO2/l (-38.66%); and 150–141.8 mgO2/l (-5.46%) for (ww25% + Pseud), (ww50% + Pseud), (ww75% + Pseud), (ww100% + Pseud), and (ww control).

The decrease in COD can be explained by the transformation of organic matter in the form of algal biomass (Nacir et al. 2010).

Reduction of total organic carbon (TOC)

Total organic carbon (TOC) is defined as any compound containing carbon atoms, except CO2, such as carbonate and bicarbonate (Chou et al. 2010; Mook et al. 2012). Implementing (TOC) analysis at water treatment facilities is a



powerful tool that can help operators continue to effectively treat water and positively affect the costs of treatment, in order to meet current and future regulatory requirements. The major compositions of dissolved organic carbon in aquaculture wastewater are humic-like substances, carbohydrates, protein-like substances, low molecular weight aldehydes, fulvic acids, phenols, and organic peroxides (Mostofa et al. 2005). Organic carbon is the energy substrate for many microorganisms. In our case study, TOC levels are gradually decreased in all samples (Fig. 9). The values of TOC were decreased as follows: from 12.5 to 3.33 mgO2/l (- 73.36%) for (ww25% + Pseud); from 25 to 10.83 mgO2/l (-56.68%) for (ww50% + Pseud); from 37.5 to 26.17 mgO2/l (-30.21%) for (ww75% + Pseud); from 50 to 30.67 mgO2/l (- 38.66%) for (ww+Pseud); and from 50 to 47.27 mgO2/l (- 5.46%) for (ww control).

These results are in agreement with the work of Houas et al. (2001) who showed that the treatment of domestic water by algae leads to a reduction of total organic carbon.

Conclusion

In conclusion, this work allows the verification of the filamentous cyanobacterium Pseudanabaena galeata strain selfpurifying capacity of dairy plant wastewater. This capacity is reflected by the increase in cyanobacterial biomass due to its adaptive potentiality to growth in this type of nutrientrich medium. In addition, it allows the reduction of COD and TOC, removal of total nitrogen, and inhibition of total bacterial flora. It should be pointed out that the dilution of the effluent before the treatment is an important factor regulating the self-purifying power of cyanobacteria. As a result, *Pseudanabaena galeata* can play a potential key role in the purification of wastewater from industrial effluents.

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