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BIOLOGICAL REMOVAL OF DAIRY INDUSTRIAL WASTES BY MARINE CYANOBACTERIA

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ABSTRACT

The possible utilization of whey by some marine cyanobacteria for growth and waste management was studied. The results indicated that both sweet and salt whey could be used as a culturing media for all the tested strains. Also, it was found that nitrogenase activities increased when such strains were grown in any of whey types. Among the tested strains, *Nostoc sp.* proved to be the most promising one for better bioremoval of whey as one of the major during industrial wastes.

Keywords: Cyanobacteria-Marine-Whey-Dairy industrial wastes-Bioconversion.

INTRODUCTION

Whey is the main by-product of both dairy industry and cheese manufacture. It is realised that for every kilogram of milk used for cheese production either 100g or 200g ends up as hard or soft cheese, respectively. The rest is whey which has to be disposed of in a manner not to cause pollution problems and/or to make use of the nutrients available in it. Worldwide production of whey was in the region of 130 million tons per year in 1992 with 3% expected annual increase thereafter *(Zadow, 1992)*. In Egypt it was reported to be about 500,000-1.000,000 tons per year *(Zaid, 1997 and EL-Gindy, 1997)* of which a large proportion is salted whey (5-10% salt) as a result of Domiati cheese manufacture.

Whey generally contains about 6 to 6.5% solids (TS), which represents almost half the total solids of milk from which is derives, these are lactose, proteins, minerals, salts, vitamins and traces of fat of which lactose is the major constituent representing from 4.5 to 5% and thus it constitutes about 70% of TS in whey.

Sweet whey results from the manufacture of products that principally use rennet-type enzymes at about pH 5-6 and contains more total solids, protein, lactose, lipids and less calcium and phosphorus contents than those of Cottage cheese whey *(Schmidi et al., 1984)*.

Salt whey contains substantial amounts of salt as sodium chloride (6-10%) when produced from the pressing of hard cheese, or when it is a by-product from Domiati cheese where salt is added to milk prior to renneting *(El-Samragy and Zall, 1988)*.

Durmus et al. (1999) investigated the effects of whey (5,10, 25, 50 and 75%) on growth of *Anacystis nidulans, Nannochloris* and *Dunaliella tertiolecta*. The composition (mg/L) of whey was: 2.75 Po_4^{-3} 43; 4-86 NO_3^- ; 0.033 NO_2^-; 41 So_4 2^-, 98 Na^+ and 11 K^+. Biochemical oxygen demand (BOD) and chemical oxygen demand (COD) were 1600 and 5434 mg/L,

respectively. The best growth parameters for the tested cyanobacteria were obtained at a whey concentration of 50, 25 and 10% for *Anacystis, Nannochloris* and *Dunaliella,*respectively. The whey had a toxic effect at higher concentrations, but had a beneficial effect at lower concentrations.

In addition, *Sharma and Mehta (2001)* studied the effect of dairy effluent on growth and biochemical composition of cells of *Oscillatoria, Aulosira Sp., Scytonema Sp., Anabaena Sp. and Tolypothrix Sp.* Cyanobacterial cells were cultured at effluent concentrations of 10, 20, 40, 50, 80 and 100%. The biochemical composition, particularly content of chlorophyll, (a) beta-carotenoids, gamma-carotenoids, protein and carbohydrates cyanobacterial cells were determined. A general increase in the biochemical composition was observed in cyanobacterial cultures. The highest concentration of chlorophyll (a) and carotenoids was recorded in cells grown in 80% effluent. Chlorophyll concentration showed 127-60% increase as compared to control. Effluent was found to stimulate the formation of beta- and gamma carotenoids.

This study was performed to find out the possibility of utilizing different types of whey by some unicellular and filamentous marine cyanobacteria for:

a) The replacement of recommended routine medium (ASN III) to reduce culturing cost.
b) The biological removal of one of the major dairy production wastes in order to reduce the effect of whey as a stream pollutant element.

MATERIALS AND METHODS

1. **Cyanobacterial strains:** Pure cultures of *Xenococcus 1, Xenococcus 2 , Pseudoanabaena, Anabaena, Nostoc, Synechocystis,* and *Synechococcus* were obtained from department of Microbiology. Fac. of Agriculture, Cairo University.
2. **Dairy industrial liquid waste:** Different whey types, were kindly provided by Milk Technology Processing Unit, Dairy Department, Fac. Agric., Cairo University. The composition of such whey is presented in Table (1).

Table (1) Composition of different types of whey.

Component, %	Sweet whey[1]	Salted whey[2]
pH	5.6-6.1	-
Water	93.2-93.6	-
TS	6.4-6.8	14.38 – 16.79
Lactose	4.9-5.1	4.62
Protein	0.8-0.9	1.01 – 1.17
Fat	< 0.05	0.72 – 0.95
Lactic acid	0.2	-
Ash	0.6-0.7	2-2.05
Salt	-	6.8

1. Moidler et al. (1980).
2. El-Samragy et al. (1988).

3. **Maintenance media and culturing conditions:** All cultures obtained are maintained under photoautotrophic growth conditions in ASN III. medium. Both enrichment and stock cultures are grown under continuous illumination, with Philips Fluorescent white lamps, at a relatively low light intensity (400-500 Lux) and incubated at 30°C.
4. **Enumeration of bacteria:** Plate count technique was employed for detection of bacterial numbers present in cyanobacteria cultures in different applied treatments. One ml of each culture was suspended in nutrient broth and serially diluted to 10^{-4}. One ml samples of each dilution were added to nutrient agar medium which were then plated and incubated at 30°C for 1-3 days to allow for bacterial growth. Results are recorded as cells numbers/ ml culture.
5. **Measurement of N_2-fixation for cyanobacterial cultures:** N_2-fixation efficiency for cyanobacteria strains was measured applying the Acetylene Reduction Activity (ARA) assay ***(Stewart et al., 1967 and 1968)***, in flasks containing all cyanobacterial cultures grown in either ANS III medium or different whey media were incubated at 30°C for 15-30 days. Then, one ml from every flask is then transferred to 7 ml bijou bottles. The bottles were then tightly sealed with a rubber cap set by an aluminum sleeve and 1 ml of C_2H_2 is then injected in each bottle and incubated at 30°C for 2-3h. From each of the bottles 1 ml sample of gas phase is withdrawn to analyse for C_2H^4 production by gas chromatography using HP 6890 Gas chromatograph equipped with stainless steel column (2 mm x 2m) packed with propack N at 60°C and with flame ionization detector at 120°C. The carrier gas was N_2 (flow rate 30 ml min^{-1}) and air flow rate was 300 ml min^{-1}. Levels of C_2H_4 produced were quantified by measuring peak heights relative to standards. The results obtained are expressed as μ moles C_2H_4 produced ml $culture^{-1}h^{-1}$.

6. Media:

a) ASN III medium *(Rippka et al., 1979)*, contains (gL^{-1}): NACl, 25: 0; MgCl2.6$HgCl_2$ 6H_2O 2.0; KCl, 0.5; $NaNO_3$, 0.75; K_2HPO_4. 3H_2O, 0.02; $MgSO_4$. 7H_2O, 3.5; $CaCl_2$. 2H_2O, 0.5; Citric acid, 0.003; Ferric ammonium citrate, 0.003; EDTA (disodium magnesium salt), 0.005; Na_2CO_3, 0.02; Trace metal mixture, 1ml L^{-1}, Deionized water, 1000 ml; pH after autoclaving 7.5. Trace metal mixture contains (gL^{-1}): H_3BO_3,2.86; $MnCl_2$. 4H_2O, 1.81; Zn SO_4. 7H_2O, 0.222; Na_2 MoO_4. 2H_2O 0.390; $CuSO_4$. 5H_2O, 0.079; Co $(NO_3)_2$. 6 H_2O, 0.0494.

b) Nutrient agar medium: *(Oxoid, 1965)* contains (gL^{-1}): Beef extract, 3.0; peptone, 5.0; Agar, 15.0; Distilled water, 1000 ml.

c) Ashby's medium: *(Abdel-Malek and Ishac, 1968)* contains (gL^{-1}): Manitol, 10.0; Sucrose, 10.0; K_2HPO_4, 0.5; $CaCO_3$, 5.0; $FeCl_3$, 0.005; $MnSO_4$. 4 H_2O, 0.005; NaCl, 0.2; NO_2 MoO 4.2 H_2O, 0.001; $CaSO_4$, 0.1; Distilled Water, 1000 ml; pH, 7.

Experimental:

10 ml of 30 days old cyanobacterial cultures were cultured in 250 ml conical flasks containing 100 ml whey or 100 ml ASN III medium and subjected for different culturing conditions and treatments as follows:

1. ASN III medium only.
2. ASN III medium + sweet whey (1:1 v).
3. Complete sweet whey.
4. ASN III medium + salt whey (1:1v).
5. Complete salt whey.
6. Whey liquid only with both types and concentrations.

Whey of both types was diluted with ASN III medium (1:1 v) for both treatments 2 and 4.

Relative growth for all strains in all treatments were examined micro-scopically and results were recorded after 10-15 days incubation on 30°C.

RESULTS AND DISCUSSION

Data presented in Table (1) indicated that the sweet whey contains 0.8-0.9% protein and 4.9-5.1% lactose. While, such contents did not exceed 1.01–1.17% and 4.62%, respectively, in salted whey. These results indicate that both whey types are good source for both nitrogen and carbon sources required by different microorganisms. Similar results were obtained by *Abou El-Khair (2002)* as the used whey contained 0.62% protein and 5% lactose.

Regarding the utilization of whey by the tested cyanobacterial strains, results in Table (2) revealed that both non sterilized salt and sweet whey exhibited better growth than that observed in both sterilized whey types. *Sharma and Mehta (2001)* reported similar trends when they indicated that dairy effluent stimulates the growth of cyanobacteria and increase its nutritive value. Also it is demonstrated that no considerable differences was found in growth of all cyanobacterial cultures when grown in either diluted whey types.

Table (2): Relative growth of cyanobacteria in different types of whey.

Strains	Sweet whey		Salt whey		Diluted whey	
	Sterilized	Non-Sterilized	Sterilized	Non-Sterilized	Sterilized	Non-Sterilized
Xenococcus Sp.1	+	++	+	++	+++	+++
Xenococcus Sp. 2	+	++	+	++	+++	+++
Pseudoanabaena Sp.	++	+++	++	+++	+++	+++
Anabaena Sp.	+	++	+	++	++	++
Nostoc Sp.	+	++	+	++	++	++
Synechocystis Sp.	+	++	+	++	++	++
Synechococcus Sp.	+	++	+	++	++	++

+ , weak; ++ good; +++, dense growth.

In respect to bacterial count associated with cyanobacterial strains when being cultured in different media, data in Table (3) indicated that the lowest bacterial count was 11-41 x 10^2/ml culture and was recorded when such strains were grown on ASN III medium. Also, results show that bacterial densities sharply increased when cyanobacterial strains were cultured in either diluted or complete whey media types. For example and irrespective of

whey type, 250-1200 x 10^2 and 200-980 x 10^2 bacterial cell/ml culture were estimated in diluted and complete whey media, in that order. Such increases could be, to some extent, attributed to the natural microbial content, i.e., 300-720 x 10^2/ml culture found in both control whey media.

Table (3): Total bacterial counts (x 10^2 /ml culture) in cyanobacterial cultures after 72 h incubation in different culturing media and whey.

Strain / Treatments	ASN III medium	Sweet whey		Salt whey	
		Diluted (1:1)	Complete (100%)	Diluted (1:1)	Complete (100%)
Whey medium	-	720	300	315	610
Xenococcus Sp.	24	420	200	600	400
Xenococcus Sp.	41	910	510	1200	540
Pseudoanabaena Sp.	11	500	280	680	280
Anabaena Sp.	36	480	980	1200	800
Nostoc Sp.	29	1200	400	720	280
Synechocystis Sp.	16	360	220	250	290
Synechococcus Sp.	22	380	200	600	260

The influence of cyanobacteria growth for 2 and 4 weeks in different whey types on their nitrogenase activities was evaluated. Data in Table (4) revealed that after 2 weeks incubation and irrespect of the tested strains, the highest averages of (ARA) *e.g.* 330.1 and 302.6 µ mole C_2H_4 m^{-1} $culture^{-1}$ were recorded when the ASN III and diluted salt whey were used as culturing medium, respectively. In addition and regardless the applied culture media, both *Nostoc* and *Pseudoanabaena* strains exhibited the highest average rates in nitrogenase activity, the ARA values in their cultures after 2 weeks incubation reached the averages of 349.9 and 322.5 µ moles C_2H_4 ml^{-1} $culture^{-1}$, in that order. On the other hand, after 4 weeks incubation and irrespect of the examined cyanobacterial strains, the complete salt whey was the superior among the tested culture media resulting in the highest averages of ARA *i.e.* 423.5 µ moles C_2H_4 ml^{-1} $culture^{-1}$. Similar to that previously observed and regardless of applied media, *Nostoc sp.* strain recorded the highest ARA after 4 weeks with an average of 396.8 µ moles C_2H_4 ml^{-1} $culture^{-1}$.

In this respect, *Thangaraj and Kulandaivelu (1994) and Durmus et al. (1999)* reported that the cyanobacterium *Anacystis* cells showed 70-90% growth in the dairy waste water and produce 50-60% H_2 when compared to these grown in the normal culture medium.

However, the results may indicate that the dilution of salted whey before using as a culture medium led to relative stimulation in nitrogenase activities for tested cyanobacteria strains after 2 weeks of incubation. This is most likely due to decreasing the total N amount and subsequently induced these strains to a better growth and enzymatic activities.

Table (4): Acetylene reduction activity (μ mole C_2H_4 / ml culture/ h.) for cyanbacterial strains after 2 and 4 weeks incubation in various culturing media and whey.

Strains / Treatments	2 weeks culturing in					4 weeks culturing in				
	ASN III medium	Sweet whey		Salt whey		ASN III medium	Sweet whey		Salt whey	
		Diluted (1:1)	Complete (100%)	Diluted (1:1)	Complete (100%)		Diluted (1:1)	Complete (100%)	Diluted (1:1)	Complete (100%)
Whey medium	-	94.3	307.0	233.6	101.6	-	172.6	207.9	356.9	282.2
Xenococcus Sp.	285.6	298.2	264.0	365.4	185.8	184.4	321.3	377.4	239.0	-
Xenococcus Sp.	302.8	221.5	261.6	252.2	164.3	142.4	280.2	266.2	124.7	-
Pseudoanabaena Sp.	325.6	287.2	236.5	293.0	469.2	298.1	345.0	292.9	291.2	239.7
Anabaena Sp.	478.3	267.5	287.2	270.6	193.6	218.9	277.6	312.8	169.2	325.0
Nostoc Sp.	534.7	267.5	-	321.6	193.4	316.7	185.1	345.2	263.4	872.9
Synechocystis Sp.	197.6	285.0	261.7	305.2	37.9	78.7	570.2	453.2	-	294.6
Synechococcus Sp.	184.7	297.8	198.8	-	-	96.82	380.2	325.4	-	527.5

Also, it could be concluded that ARA averages recorded for all strains, at the same period, in the diluted salt whey were higher than those observed in their correspondings in the diluted sweet whey. This in presumably reasonable since the tested strains are marine and they do require a relative salinity for growth and activity *(Mackay et al., 1983 and 1984)*.

In general, the results may demonstrate the possible use of whey to sustain the growth of cyanobacteria representing a relatively inexpensive and efficient substrate medium for growth and activity of cyanobacteria.

It may be also concluded that cyanobacteria could be considered a suitable agent for the bioconversion and/or removal of whey and dairy effluent in respect to dairy waste management.

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التخلص الحيوى من المخلفات السائلة لمصانع الألبان باستخدام السيانوبكتريا البحرية

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أجرى هذا البحث لدراسة إمكانية استخدام بعض سلالات السيانوبكتريا البحرية للشرش الناتج من مصانع الألبان لتنميتها وكذلك كوسيلة للتخلص من الشرش كأحد مخلفات مصانع الألبان.

وقد أشارت النتائج إلى أن كل من الشرش المالح والشرش الحلو يمكن استخدامهما كبيئة مناسبة لتنمية جميع سلالات السيانوبكتريا البحرية المختبرة. كذلك دلت النتائج على أن نشاط أنزيم النيتروجينيز لنفس السلالات زاد عند تنميتها على أى من نوعى الشرش. من ناحية أخرى أثبتت سلالة *Nostoc sp.* أنها الأفضل بين السلالات المستخدمة فى قدرتها على التخلص الحيوى من الشرش أحد أهم مخلفات مصانع الألبان.