Effective Production of Bacterial Cellulose Using Acidic Dairy Industry By-Products and Agro Wastes

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Several studies are conducting worldwide to produce cellulose by using processes that reduce both environmental impact and production cost. Cellulose, which can be produced by several sources, is one of the most abundant macro-molecules on Earth. Although its production is mostly by vascular plants, an alternative route that not only replaces but also reduces the demand from plants is the production of cellulose from another resource such as a bacterial system. The main objectives of the present study were first to evaluate BC production by some specialized bacteria isolated from spoiled fruits (agro wastes) using milk whey or dairy wastewater as media without any supplementation and verify the effects of this culture media on BC structure. Second, it aimed to evaluate bioremediation ability of the isolated bacteria for purification of polluted wastewater. Three exogenous bacterial isolates were isolated from Tomato (T0) and Pomegranate (R01 & R02). Raw dairy wastewater samples were collected from Milk Factory in Alexandria governorate, Egypt during the course of the study. The 3 bacterial isolates were investigated for efficient cellulose production using acidic dairy effluent as their sole nutrition growth medium as well as their ability to purify the highly polluted dairy wastewater and milk whey. They were used as free-living individual and mixed cultures in batch mode bioassays. Wastewater quality parameters included Temperature, pH, TSS, TDS, BOD, COD and bacterial TVC were determined before and after treatment and the removal efficiencies was calculated. In addition, the yield of cellulose was determined.

The 3 exogenous bacterial isolates T0, R01 & R02 were molecularly identified as *Bacillus velezensis* strain FZB42 (T0), *Klebsiella quasipneumoniae subsp. similipneumoniae* strain 07A044 (R01 and R02). Production of BC by the selected bacteria using different media revealed that:

A. Cellulose was detected as white pellicle covering the surface of HS liquid medium but was very costly.

B. No cellulose production was detected using tomato juice as growth medium.

C. The raw milk whey did not stimulate the required enzymes responsible for cellulose production.

D. Raw dairy wastewater could efficiently provide the tested cultures with all nutrients requirements that stimulate the production of significant amount of cellulose.

E. The maximum BC yield recorded 31.9, 25.8, 25.58 and 23.4 g/L achieved by *B. velezensis* strain FZB42 (T0), *K. quasipneumoniae* (R02), bacterial consortium of the 3 strains and finally *K. quasipneumoniae* (R01), all after 15 days under static conditions and at room temperature using raw highly polluted dairy wastewater as growth medium.

F. These BC amounts are much higher than any of the mentioned levels by other bacteria proving the excellent advantage that recommend their use for this purpose.

G. The maximum yield of BC by strain T0 is 2.42- fold higher than that produced by the standard HS medium (13.18 g/ L).

Bioremediation of the dairy wastewater using the selected bacteria revealed the following results:

A. Raw Dairy wastewater contained high levels of TSS (1720 mg/l) and TDS (1500 mg/l) as well as much higher levels of COD and BOD (10000 and 3800 mg/l) respectively.

B. All strains showed highest RE of the TSS, TDS, COD and BOD reaching RCs of 12, 2050, 75 and 25 mg/l respectively. All the achieved RCs for the tested parameters are compiling with the law with much lower levels than their MPLs (60. 5000, 80 and 60 mg/l respectively).

C. Therefore, they are efficient strains for the treatment of the dairy wastewater as individual and mixed culture.

D. The 3 selected exogenous bacteria R01, R02 and T0 showed growth stimulation during the batch treatment bioassay. T0 showed the highest growth stimulation recording 7.78-fold higher growth density than its initial density. This was followed by R01 and R02 with 1.78 and 1.75-fold higher growth stimulation compared to their initial densities respectively after 8 exposure days.

E. On the other hand, the mixed culture and the raw dairy influent (control) exhibited high growth inhibition recording 83.33 and 82.75% respectively during the treatment duration (8 days) indicating their sensitivity to the raw influent contents or the biodegradation by-products or secondary metabolites produced during the treatment process.

Bioremediation of the milk whey using the selected bacteria revealed the following results:

A. Remarkably higher COD levels were detected in the raw milk whey (60000 mg COD /l) at the starting point indicating huge organic load.

B. The Mixed culture exhibited the highest removal efficiency (62%, RC: 22800 mg/l) followed by R01 & R02 (58 and 56.83%) equivalent to RCs of 25200, 25900 mg/l respectively and finally T0 (42.66%, RC: 34400 mg/l) all after 30 days.

C. Although reasonable removals were achieved the RCs of the COD still far exceeding the MPL of 80 mg/l stated by the law. The lowest achieved RC recorded 22800 mg/l which is 285- fold higher than its MPL.

Batch Mode Production of Cellulose Using Bacterial Biofilms and Dairy Wastewater

A. No cellulose production was detected by any of the tested bacterial biofilm systems along the experiment duration (5 months).

B. Bacterial biofilm showed very clear decontamination of dairy wastewater shown as reduction of turbidity that increased with time indicating degradation of the included pollutants.

Surprisingly, much higher BC was produced by all the tested bacteria when grew in the raw dairy wastewater compared to the HS standard medium. This is considered as an excellent achievement and value-added technology for the feasible production of BC on acidic dairy wastewater. Therefore, the proposed BC production technology used in the present study is highly recommended to be scaled up to industrial or semi-industrial application.

Results also proved that using the isolated exogenous bacteria as individual or mixed culture for the treatment of dairy effluents is remarkably efficient since it reduces the treatment time and hugely increased the removal efficiency of all the tested parameters due to increasing the growth of the living bacteria. Therefore, it is highly recommended to use the selected bacteria for dual benefits; BC feasible production and purification of the highly polluted dairy wastewater.