REMOVING COD, N AND P FROM DAIRY WASTEWATER BY EMPLOYING *LACTOBACILLUS PLANTARUM* WITHIN MICROBIAL ELECTROLYSIS CELL

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ABSTRACT

This study explored the capability of treating real dairy wastewater using the microbial electrochemical system by using *Lactobacillus plantarum* species as a microorganism. The samples were collected from a local factory for dairy products in Baghdad. Three main parameters were investigated with different levels, for estimating chemical oxygen demand (COD), phosphate (P) and nitrogen (N) removal efficiency. The studied parameters were detention time, the potential difference between electrodes and initial concentration of COD. The respective optimum values for these parameters were 17 days, 400 mV and 17000 mg COD/L respectively. At the optimum values, the optimum COD removal efficiency was 99.4%. Meanwhile, the study also performed removal efficiency for N and P due to their effects on the aquatic life and ecosystem. The optimum removal efficiency for P and N were 99% and 99%, respectively.

Keywords: Microbial electrolysis cell, potential difference, graphite electrodes, anaerobic degradation, recirculation velocity, food safety

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إزالة المتطلب الكيميائي للأوكسجين والنايتروجين والفسفور من مخلفات معامل الالبان باستخدام بكتريا اللاكتوباسلس بلانترام ضمن خلية التحليل المايكروبي

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المستخلص

تضمنت هذه الدراسة بيان امكانية استخدام بكتريا اللاكتوباسلس بلانتارم ضمن خلية التحليل العضوي لمعالجة مخلفات معامل الالبان. حيث تم جمع كافة العينات الواقعية من احد معامل منتجات الالبان في مدينة بغداد. تم دراسة ثلاثة عوامل والتي تؤثر تأثيراً مباشراً في كفاءة ازالة المتطلب الكيميائي للاوكسجين والنايتروجين والفسفور, في حين كانت العوامل المؤثرة والتي تم دراستها هي زمن المكوث والفرق في الجهد الكهربائي بين الاقطاب واخيراً التركيز الابتدائي للمتطلب الكيميائي للاوكسجين. أظهرت نتائج التجارب المتعددة ان الظروف الامثل للحصول على اعلى كفاءة ازالة هي عندما يكون زمن الاحتجاز 17 يوماً والفرق في الجهد الكهربائي بين الاقطاب 400 ملي فولت و التركيز الابتدائي للمتطلب الكيميائي للاوكسجين 17000 ملغم/لتر. حيث كانت كفاءة الازالة ضمن الظروف آنفة الذكر 99,4% للمتطلب الكيميائي للاوكسجين و 99% للنايتروجين و 99% للفسفور على التوالي.

الكلمات مفاتحية: خلية التحليل المايكروبي, فرق الجهد, اقطاب الغرافيت, التحلل اللاهوائي, سرعة التدوير، غذاء امن.

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INTRODUCTION

Day by day dairy industry improved and developed. The main reason for this development is the tight relationship between dairy consumption and population growth (10, 11, 29, 30). Many kinds of dairy products appeared, and consequently increased the wastewater. Dairy wastewaters includes many pollutants. The highest concentrations are chemical oxygen demand (COD) and biochemical oxygen demand (BOD). Other pollutants included nitrogen (N), phosphorous (P), total dissolved solids (TDS), total solids (TS), total suspended solids (TSS), oil, and grease are also found, these are played an important role in estimating water quality, when it was discharged directly or with weak treatment to the surface water26. In particular, N and P have a great deal in dairy wastewater treatments due to their crucial effects represented by eutrophication. While; organic matter depleted oxygen concentration existed in water bodies18. The calculations of the World Bank Group cleared that meat and dairy products consume approximately 25% of the total freshwater that is used by the food and beverage industry34. These amounts were used for cleaning, sanitizing, heating, cooling, and floor washing. Traditionally, different processes were used for treating dairy wastewater. Aerobic and anaerobic were the general highlight. Activated sludge process, aerated lagoons, trickling filters, sequencing batch reactor, anaerobic sludge blanket (UASB), anaerobic filters, represented the specified techniques used37. The main disadvantages of these techniques are costly, and consume a lot of energy while producing a large quantity of sludge37. In the other hand, various physicochemical methods were modified and also used to treat dairy wastewater. Coagulation or flocculation is the most commonly used one (37). In general, dairy wastewater was pre-treated using various inorganic or organic coagulants, followed by filtration, e.g., nanofiltration (NF) or reverse osmosis (RO)(6, 17). Microbial electrochemical systems (MESs) are considered rapid development technologies in the sector of water and energy (25). MESs were studied intensively and modified greatly in the last decade; it integrates many scientific fields like microbiology, electrochemistry, materials science, engineering, and many related areas together (39). The main features of MESs are providing not only a solution for environmental problems, but giving a big chance to understand sensitive microbial electrochemistry; and opening different platforms for engineering functions. It is interesting to know that all the MESs share one principal role, degrading (oxidizing) different substrates (wastes) at the anode by the microorganisms to generate electrical current39. The generated electrical current is either captured and reused as electrical energy, which can be noticed clearly in a microbial fuel cell (MFC) (27), or exploited to produce different valuable gaseous such as H2, H2O2, CH4 and other different compounds, which can be shown in microbial electrolysis cell (MEC) and microbial electrosynthesis cell (MSC) (35). Other purposes may also be achieved such as; remediating contaminants as in remediating microbial cell (MRC), or desalinating purposes as in microbial desalinating cells (MDC) (39)_. In 1911 Potter reported the ability of bacteria to transport electrons between their cells and the solid surfaces19. The cascading research have shown two mechanisms for this transporting; Direct Electron Transfer (DET), and Mediated Electron Transfer (MET)8. Microbial electrolysis cell principals and concepts were started at 200539. It can be defined as a system with an external power source that increases abiotic cathode reduction during anaerobic conditions (32). This study was aimed to investigate the ability of MEC to treat dairy wastewater, particularly the removal efficiency of COD, P, and N pollutants. In addition, it proves the integration of *Lactobacillus plantarum* within the system. Altogether, this study investigated three parameters: Detention time, potential difference between electrodes, and initial concentration of COD.

MATERIALS AND METHODS

Raw material: Wastewater was collected from a holding tank of a dairy factory in Camp Sara, Al-Rusafa, Baghdad, Iraq. Samples were picked up weekly using one-liter dark bottles, and then collected as and when needed. At the factory, the wastewater was stored in a holding tank only before being discharged into the

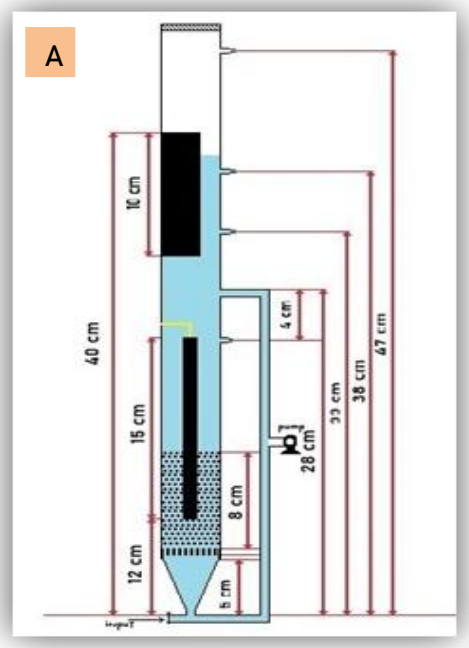
public waterway. Before laboratory analysis, some parameters are measured directly such as Temperature, pH and dissolved oxygen (DO) others are measured in the laboratory such as COD. All the picked samples were stored in dark glass bottles at 4 0C to stop all relevant biological reactions that might affect the results. A wide range of fluctuations for COD, P, and N in the wastewater were recorded. The COD values of these samples varied from 16,019 to 37,998 mg/L. The most frequent reading, i.e., 13,980 mg COD/L, which is used in this study. The concentration of P ranged from 0.9 - 0.5 mg/L and the most frequent value, i.e., 0.5 mg/L, was used for further analysis. Meanwhile, the concentration of N varied from 1.3 - 0.4 mg/L and the most frequent value, i.e., 0.4 mg/L. (Table 1) shows the main primary characteristics of dairy wastewater for the factory.

Table 1. Main characteristics of dairy wastewater

No.	Parameter	Value
1	pH	6.83
2	BOD5 (mg/L)	11505
3	COD (mg/L)	34650
4	Total suspended solids (mg/L)	730
5	Total dissolved solids (mg/L)	3667
6	Oil and grease (mg/L)	944
7	Nitrogen (mg/L)	0.4
8	Phosphorous (mg/L)	0.5
9	Iron (mg/L)	0.3
10	Turbidity (NTU)	3080
11	Electrical conductivity (µs/cm)	7640

Design of reactor

The batch experiments were conducted in a cylindrical glass reactor (4 cm in diameter and 50 cm in depth), the reactor has an input gate controlled by a valve as an entrance for the raw material and many outputs for the treated wastewater. For recirculation purposes the reactor had an entrance at the bottom. Figure 1 A and B, show the schematic and real design of the reactor.



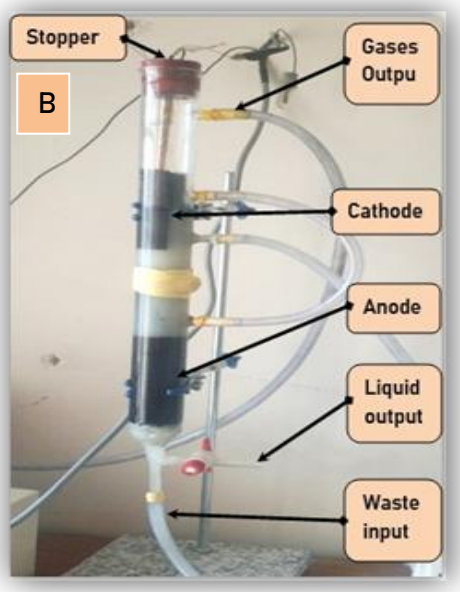


Figure 1. (A) The schematic design of MEC reactor. (B) The real design of MEC reactor

The anode was made from granular activated carbon (Purolite AC20G, UK. Size (0.6-1 mm)). The total depth of the GAC was 8.4 cm with volume equal to 105.55 ml. The particles were washed by 1 M HCl and 1 M NaOH respectively38. The particles are set over a glass mesh with holes have 0.5 mm in diameter. While the cathode were made from pure graphite (Olmec Co. Sheffield, UK). The depth of the cathode was 10 cm and the thickness was 1 cm, while the width was 8 cm rounded with the cylindrical wall. The cathode was punched and connected to titanium wire.

Titanium had the ability to carry the charges effectively with no toxic compounds that may inhabit the microorganism's growth20. A current collector was used also to harvest all the generated electrons. The current collector was made from pure graphite as a beam with 1 cm diameter and 15 cm length. The graphite beam also bunched at the top and connected to titanium wire. A reference electrode (Ag/AgCl3) was used adjacent to the current collector. A potentiostat equipment (WENKING M Lab, Germany) was adopted. A peristatic pump (LongerPump BT300-2J, China) have been set for the recirculation purposes. COD values were measured using a photometer (model: Lovibond 2420722 Vario HR-COD VARIO tube test 0 – 15,000 mg/L). The concentrations of P and N were measured using ion chromatography (Cecil, 2013, UK). Figure 2 shows some parts of the cell

Figure 2. Some parts of the MEC. (A) Cathode electrode. (B) Current collector

Microorganism selection and preparation

A standard species of *Lactobacillus plantarum* was used. The code of used microbe was "*Lactobacillus plantarum* ATCC 14917". This species was gained from the Biotechnology department, College of Science, Baghdad University, Baghdad, Iraq. It considered as safe and probiotics bacteria. To start the microorganism activation process, a suitable flask was used to dissolute 5.515g Lactobacillus MRS Broth (MRS broth granulated, GM369-500G, Himedia, England) in 100 mL of distilled water, pH=6 at room temperature. The solution then distributed equally over 10 vials, and sterilized by autoclave at 121 ºC for 15 min. After sterilization, a suitable inoculum from the *Lactobacillus Plantarum* ATCC 14917 was added to each vial, and closed tightly to achieve anaerobic conditions. The vials then incubated 2 days in incubator at 37 ºC. The growth of bacteria was noticed easily after 2 days and it was measured by spectrophotometer (Germany) at wave length 600 nm, and the growth density was 1.6, which is considered good. For acclimation purposes, an inoculum 2% of activated bacteria was added to the raw material in a suitable flask, and sealed tightly to provide anaerobic conditions, and then ncubated for 2 days at 37 ºC. The activated bacteria are then ready for use. Figure 3 shows some activation steps.

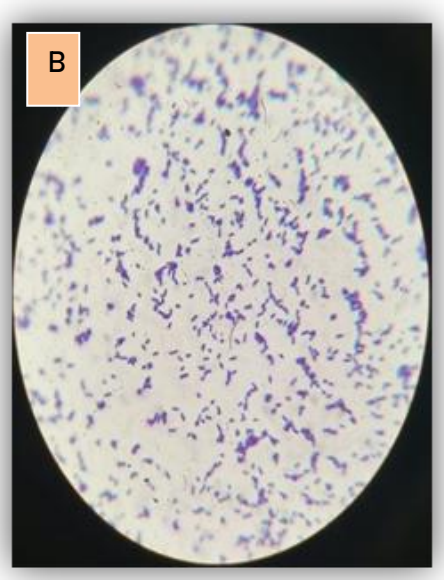


Figure 3. (A) *Lactobacillus plantarum* growth after 2 days incubation at 37 ºC in anaerobic conditions. (B) *Lactobacillus plantarum* examination under microscope with 100X

Experimental work

The experimental work was started after the acclimation process end. A net volume of raw material was added to the reactor untill it reached 38 cm height (approximately 477.52 mL with pipes volume). Inoculums of activated acclimated bacteria were added to the reactor with percentage 2% (v/v). A sample was picked up at the beginning to measure COD, P and N. Recirculation was set at an upward velocity 0.265 cm/sec.

The anaerobic conditions were gained by closing all the outlets and connecting a balloon at gases output. Three parameters were examined as follows; detention time, potential difference and initial concentration of COD. All experiments were conducted at room temperature. The optimization process then started for the chosen parameters. The first parameter that was examined detention time at a fixed potential 200 mV and COD concentration equal to 13980 mg/L. Second optimized parameter was the potential difference. Three main levels were studied (200, 400 and 600 mV). While the final parameter was initial concentration of COD, and the studied levels was (2000, 17000, 30000 mg/L). Readings for current and resistance were measured every 6 hours. Samples were picked up daily for measuring COD, P and N. removal efficiency was calculated according to Equation 133:

COD Removal efficiency = (C0-C)/C0 100 ……. 1

Where C0 is the initial (COD, P or N) concentration (mg/L) and C is the final (COD, P or N) concentration (mg/L) at the end of the experiment

It is worth mentioning that a separate experiment was done for the raw material without using MEC system. The experiment was done by fluidizing and mixing the raw material with active bacteria in anaerobic conditions inside the reactor.

MEC mechanism

Many researches were agreed that to achieve efficient microbial electrochemical system, anode or cathode potential should be maintained at the optimum level41. The system which designed and used for this purpose was called 3-electrode microbial electrochemical cell (3D-ME)31. Two main possibilities can be achieved, optimum anodic potential (when the working electrode potential was set by using potentiostat and reference electrode to achieve optimum anodic efficiency), or optimum cathodic potential (when the potential of working electrode was set to achieve optimum cathodic efficiency). In cathodic optimum configuration, the main target was to enhance bioelectrochemical electron-accepting reaction31, by adding an external power to drive these reactions when the working electrode was set at optimum conditions, and to stimulate the microorganism's oxidation reactions. MEC is an anaerobic biological system, which usually used to product hydrogen or methane or to treat different pollutants, by degrading organic compound12. It resembles another technology for producing hydrogen: the microbial fuel cell (MFC). The main difference between two technologies was MEC produces hydrogen by anaerobic electroactive bacteria and applied voltage, whereas MFC produces an electrical current from aerobic electroactive bacteria1. The process was started when the anode (or current collector) and cathode are connected to power supply (potentiostat) and submerged in an electrolyte solution, to enhancing a microorganisms stimulation and electron transportation in the anaerobic container12. The microbes were begun to oxidizing the organic matter and liberating electrons and

protons. The electrons and protons were transport either by the current collector or solution recirculation until it reached the cathode and forming chemical compounds27. The power added by potentiostat was for enhancing production rate14. Many researches were agreed that the optimum voltage to be applied was between the range of (0.5–1.0 V)22 others suggest (0.2-0.8 V)16, which is lower than that required voltage for water electrolysis (1.23–1.8 V) (15).

RESULTS AND DISCUTION

The first step was degrading dairy wastewater by *Lactobacillus plantarum* ATCC 14917 that cultivated over granular activated carbon in an anaerobic condition within the reactor, without adding any external power. The only effect was the recirculation process at an upward velocity equal to 0.265 cm/sec, pH =6, microorganisms inoculum was 2% (V/V), granular activated carbon was 8.4 cm in height and all are set at room temperature. The initial COD concentration was 13980 mg/L, and it reached to 560 mg/L after 24 days (this concentration was allowed to be discharged to municipal sewage network according to Iraqi legislations). The removal efficiency of COD reached to 95.99% as illustrated in Figure 4.

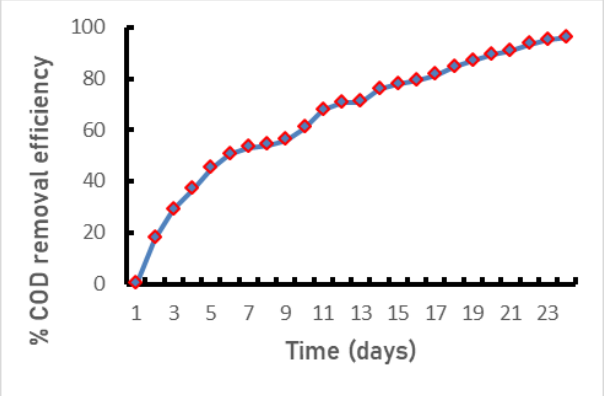


Figure 4. COD removal efficiency for the dairy raw wastewater, no external power was added. Initial COD concentration was 13980 mg/L.

Athanasia *et al*, in their work, used *Chlorella sorokiniana* to utilize dairy wastewater. They were achieved to 91% removal efficiency for COD24. While, Jürgensen and his group were used two methods; continuous stirred tank reactor and anaerobic baffled reactor to treat raw dairy wastewater. The initial COD concentration have been used in their experiment was (1300-4500 mg/L), while the removal efficiency was 91% during approximately 10 days for both methods (13). For the nitrogen and phosphorous, the concentrations within the raw wastewater was 0.4 and 0.5 mg/L respectively. The low concentrations was due to cleaning regime in the factory, which was depended on vapor system for sanitizing the main equipment. The main factor in removing N and P from dairy wastewater was the existence of tight anaerobic condition in the reactor (42).

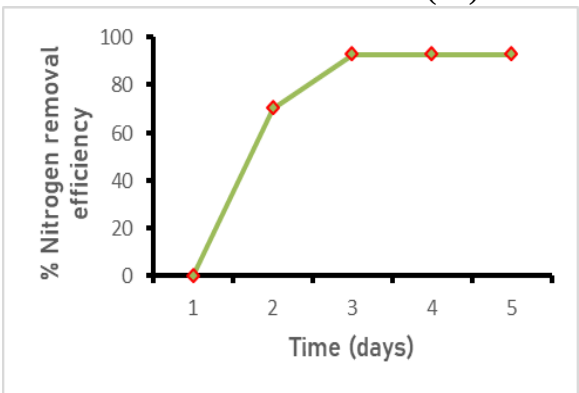


Figure 5 shows that the optimum removal efficiency of N was 92.5% after 3 days. The main reasons are exploited the nitrogen by Lactobacillus plantarum and the anaerobic conditions in the reactor which accelerate the denitrification process

(Figure 5). Nitrogen removal efficiency for dairy wastewater, no external power was added. Initial concentration of N was 0.4 mg/L. Bae et al., reached approximately 96% removal efficiency for N by studying membrane sequencing batch reactor in treating dairy wastewater2. Figure 6, illustrate P removal efficiency within the experiment. The optimum value for P removal was 82%, after two days of the experiment.

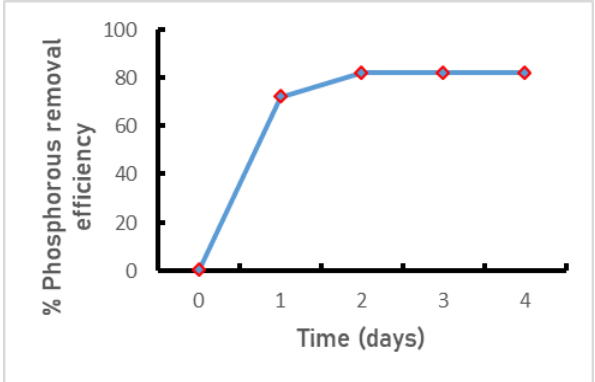


Figure 6. Phosphorous removal efficiency for dairy wastewater, no external power was added. Initial concentration of P was 0.5 mg/L

Bae *et al*., gained in his work removal efficiency for P until 80% by using sequential biological reactor with mixture bacteria, and within 36 hrs2. While, Wang *et al*., reached to 80% removal efficiency for P by using anaerobic moving bed biofilm reactor (AMBBR) techniques (39). The anaerobic degrading for the dairy wastewater by using *Lactobacillus plantarum* ATCC 14917, without using an external power was important to compare with MEC. The next step was using MEC in degrading dairy wastewater. All the configurations used in previous experiment were set identically in MEC experiments. Initial COD concentration was 13980 mg/L, upward velocity 0.265 cm/sec, pH =6, microorganisms inoculum 2% (V/V), granular activated carbon height was 8.4 cm and finally all are at room temperature. The external power used was equal to 200 mV. The first parameter examined was detention time. The optimum COD removal efficiency was 95.63% after 17 days, as shown in Figure 7.

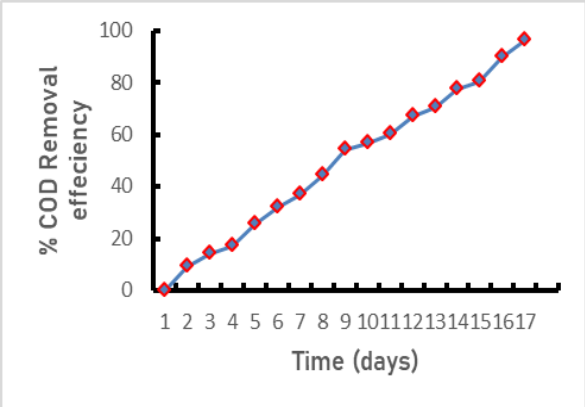


Figure 7. COD removal efficiency for dairy wastewater. External power used was 200 mV. Initial COD concentration was 13980 mg/L

Shen *et al*. have been used MEC to treat wastewater of cornstalks, the COD removal efficiency reached in this experiment was 80.2 %, voltage used 1.2 V, COD concentration 2000 mg/L and the retention time was 16 hr36. Tejedor-Sanz *et al*. showed that the removal efficiency for COD was 87% when using brewery wastewater as a feed and MEC as a treatment method. COD concentration was 1150 mg/L, detention time was 2.4 days and used voltage of 200 mV (38). Nitrogen removal efficiency was 96.3 within two days and no trace at the third day, as shown in Figure 8. Li *et al*. achieved removal efficiency for nitrogen until 85% by using MEC and three ion exchange membranes, the applied voltage was 2000 mV and COD concentration was 314 mg/L21. In the work of Tejedor-Sanz *et al*. no trace of nitrogen was recorded in the effluent of MEC process, the main reason was using the nitrogen by the microorganisms (38).

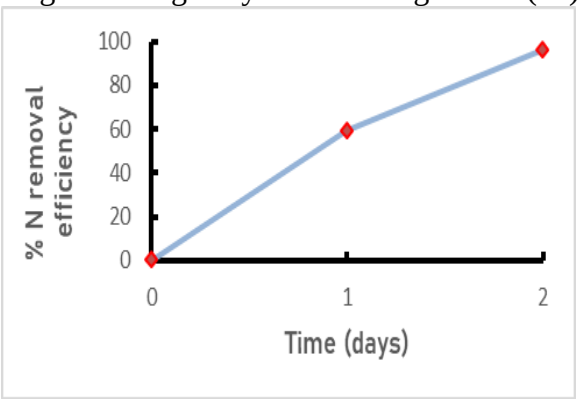


Figure 8. N removal efficiency with time. External power 200 mV, detention time 17 days, initial concentration was 13980 mg/L

The removal efficiency for P was 88% after three days and no trace at the fourth day, as illustrated in Figure 9.

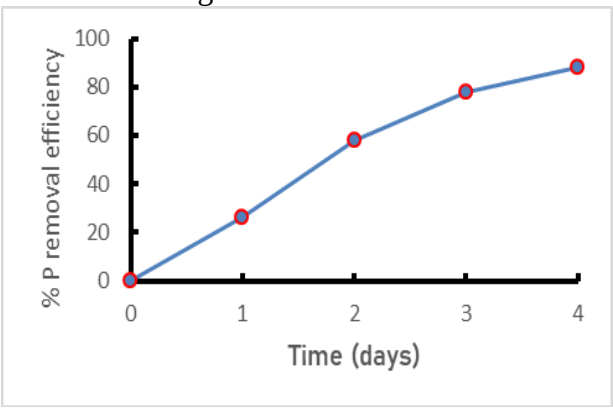


Figure 9. P removal efficiency with time. External power 200 mV, detention time 17 days, and initial COD concentration 13980 mg/L

Tejedor-Sanz *et al*. gained no trace for P after 2.4 days and voltage equal to 200 mV by using MEC38. While Li *et al*. reached to 85% removal efficiency for P, by using MEC and three layers of ion exchange membrane (21). The second examined parameter was external power added to the cell. Three main levels were studied (200, 400, 600 mV). The optimum COD removal efficiency was 99.2% achieved at 400 mV at 17 days and for initial COD concentration equal to 13980 mg/L, as shown in Figure 10.

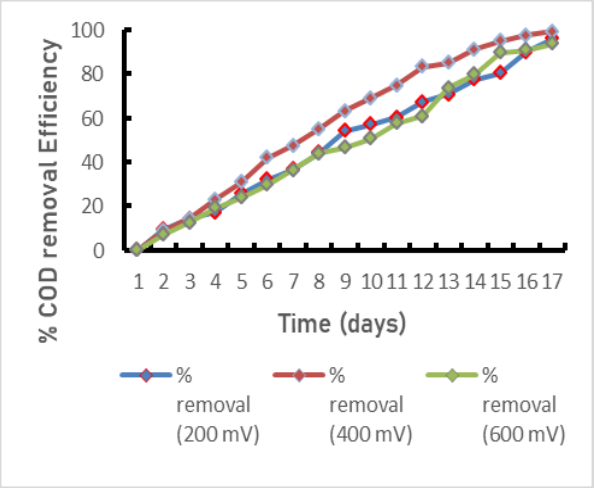


Figure 10. COD removal efficiency with time and different external power used. Initial COD 13980 mg/L, detention time 17 days

COD removal efficiency at the other voltage was higher than 90%, which give a good indicator for the microorganisms exploited and external current used. Shen et al. showed that COD removal efficiency fluctuated when using different voltages. It reached to more than 77% at 600 mV and then decreased until reached more than 90% at 1.2 V36. Lim *et al*. (22) examined the performance of MEC over different voltage magnitudes, (0.2-2 V). The results of his work stated that the minimum voltage required for the system was 0.3 V, and at this value, the anode contributed in approximately 99% of the total current22. A study have been reported that when the applied voltage is fixed at 0.3 V this will enhance the diversity of methanogenic, otherwise increasing voltage to 0.6 V will decrease microbial diversity then the activity decreased7. For the nitrogen removal efficiency, it reached to optimum value at the three voltage, and it reached 98% at 400 mV after one day. This result was agreed with that gained by Cecconet *et al*., which achieved removal efficiency for N as NO3 equal to 96.3% after 32 hrs4. Liu et al., reached to removal efficiency equal to 95% for nitrogen by using synthetic contaminated wastewater and two chambers MEC for less than one day (23). The removal efficiency of P was reached to 99% after one day at 400 mV, and there is no trace in the second day. Cusik *et al*. declared that P removal increased when the applied voltage increased21,5. Tejedor-Sanz *et al*. gained no trace for P after 2.4 days and voltage equal to 200 mV by using MEC (38). The last parameter studied was initial concentration of COD. Three main levels were examined (2000, 17000, and 30000 mg/L). Figure 11 shows the removal efficiency of COD at 400 mV and 17 days as detention time.

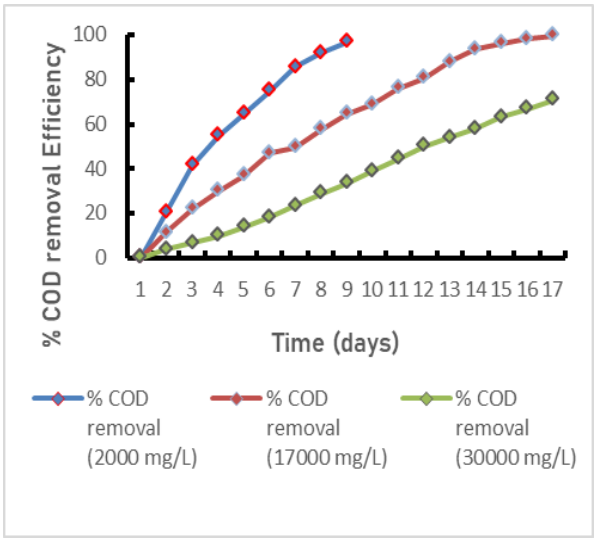


Figure 11. COD removal efficiency with different initial COD concentrations. External power used 400 mV, and detention time equal 17 days

The optimum COD removal efficiency was 97.4 %, after 17 days at 400 mV. While the removal efficiency of COD that appeared at initial concentration, 2000 mg/L was reached 96.6% at 400 mV and after 9 days. In contrast the COD removal efficiency of 30000 mg/L was 70.77% at 400 mV and 17 days. Many studies were investigated the relation between initial COD concentration and COD removal efficiency by using MEC, the examined range was below 1000 mg/L until 122300 mg/L, while the removal efficiencies ranged between 29% to 100%9. Tejedor-Sanz *et al*., achieved 87% COD removal efficiency, for 2.4 days, initial COD concentration 1150 mg/L and 200 mV as an external voltage38. Rani *et al*., have been achieved removal efficiency equal to 95% after 2 days and 0.8 V, and 18,57gCOD/L28. the same study have been showed that removal efficiency of COD decreased when initial concentration of COD increased, the removal efficiency dropped from 88% to 70% when the COD changed from 2000mg/L to 2389 mg/L28. The Nitrogen removal efficiency reached to 99% for less than one day when using 17000 and 30000 mg COD/L as initial concentration.

While the 2000 mg/L has no trace. Bae *et al*. (2), achieved 96% removal efficiency for N in 36 hrs2. While the removal efficiency for P reached to 99% at 17000 mg COD/L and 30000 mg COD/L, while the removal efficiency for P had no trace for concentration 2000 mg COD/L.

CONCLUSION

In this study, the dairy wastewater was treated using the MEC. 3D electrodes were used, granular activated carbon as an anode, and graphite rounded as cathode and current collector. The experiment proved the ability of *Lactobacillus Plantarum* AC 14917 to degrade real dairy wastewater with removal efficiency reached to 99.4%. Different parameters were studied within the experiments in three levels detention time, potential difference between electrodes and initial COD concentration. The optimum conditions for the MEC treatment were: detention time 17 days, difference potential between electrodes was 400 mV and initial concentration of COD 17000 mg/L. The MEC treatment achieved removal efficiency, 99% for phosphorous and 99% for nitrogen. The large surface area of the granular activated carbon and the type of bacteria fueled the high removal efficiency.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

DECLARATION OF FUND

The authors declare that they have not received a fund.

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