نجربة تلميذ مبدع

Bacillus Subtilis bioremediation: COD and pH adjustment

- By:

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◙ ملخص المشروع :

نظرًا لعدم الإدارة الجيدة للمياه، وتزايد عدد السكان ، والنمو الاقتصادي السريع في دول حوض النيل؛ فإن مستوى المياه في البلاد ينخفض بشكل مطرد.

ويمكن ا ستخدام المياه في العديد من المناطق لتلبية احتياجاتنا المنزلية، وإنتاج العنا صر المختلفة التي نحتاجها في حياتنا اليومية ، وري النباتات اللازمة للبقاء على قيد الحياة، وأحد أهم الحلول لـ شكلة المياه في م صر هو إعادة ا ستخدام المياه في العمليات المختلفة التي تنتج مياهًا ملوثة، فالغرض الرئيس من هذا المشروع هو إعادة ا ستخدام مياه الصرف الناتجة من صناعة إعادة تدوير الورق في العملية نفسها.

وكانت مشكلة مياة الصرف تتركز في الآتي: انخفاض الأس الهيدروجيني "pH" بقيمة تـ ساوي (6.13) و بقيمة (13614) جزء بالمليون لـ . ."COD" ؛ ويرجع هذا إلى العديد من العوامل التي حدثت أثناء عملية التـ صنيع؛ لذلك تم إنـ شاء عملية معالجة حيوية با ستخدام بكتيريا Bacillus Subtitles لحل هاتين المشكلتين، وتم اختيار متطلبات التصميم الخاصة بنا لتكون الأس الهيدروجيني وال COD ، والآثار البيئية لحلنا على البيئة.

و كانت النتائج مذهلة، حيث تغير الأس الهيدروجيني إلى (٧,٦٠) و COD إلى (١٢١٧) جزءًا في المليون، علاوة على ذلك ، فإن الآ ثار البيئية لعملية المعالجة الحيوية ليس لها أي جانب سلبي.

بناءً على النتائج المذكورة أعلاه ، يمكن القول: إن الحل المقترح كان فعالًا للغاية، وخاليًا من التكلفة تقريبًا مقارنة بالحلول التي تم إجراؤها مسبقا.

◙ Abstract:

Due to various inadequate water management, growing populations, and rapid economic growth in the Nile Basin countries, the country's water level is steadily declining. Water can be used in many areas to meet our domestic needs, produce different items needed in our daily lives, and irrigate the plants necessary for survival. In order to be eligible for use, the key purpose of this initiative is to reuse the resulting wastewater from the paper industry in the same process.

The issue with the effluent was having a low pH level equal to 6.13 and a very high chemical oxygen demand "COD" value of 13614 ppm. This dates

back to several factors that came from the manufacturing process. Therefore, a bioremediation process was constructed using Bacillus Subtilis bacteria to solve these issues. Our design requirements were selected to be the pH, COD, and the environmental implications of our solution on the environment.

The results were impressive, where the pH changed to be 7.60 and the COD to be 1217 ppm furthermore the environmental implications of the bioremediation process does not have any negative side but positive side.

From the results above, it can be concluded that the solution proposed was highly effective and almost cost-free compared to previously done solutions.

Keywords: Bacillus Subtills, Bioremediation, Paper recycling Industry, Wastewater Reuse,and Water Quality.

Theme: Improving Water quality

INTRODUCTION

The need to discuss and solve Egypt's grand challenges is very crucial. As known, Egypt has faced water scarcity from the early beginning of population growth: due to the increase in water usage in the various fields of life.

Nowadays, water in Egypt is more subjected to Scarcity due to several reasons, including the latest progress of the Ethiopian dam inauguration. The improvement of the reuse of wastewater and water by-products is now essential to help stop water threats. This project is going to search upon the hidden lines of research papers and previously done projects to help find more technological solutions for those threats. DuPont made one attempt to help reusing wastewater. DuPont used the green chemistry methods to change the industrial solvents and convert tide detergents into greener ones, "cellulosic ethanol", making water reuse possible. This solution has successfully solved the problem, reduced environmental damage and increased efficiency. However, it had a relatively high cost.

The presented solution has managed to solve the pH problem associated with the wastewater of the Paper recycling industry using "Bacillus Subtilis" or B.S. bacteria ,so for the design requirements, the starting was by a simple proposal about the importance of reducing pH and COD for the wastewater, since the pH and the COD were the main issues with not only the effluent of the paper recycling industry but almost all the Egyptian

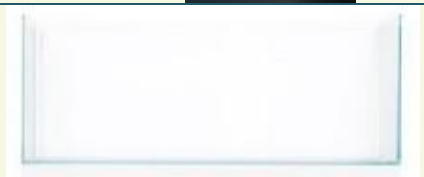
industries and even daily activities. Because of the possible danger accompanied with using a bacterial bioremediation, the environmental implications of the solution on the environment and living organisms were chosen as the second design requirement based on the project itself. Furthermore, the positive implications of the B.S. bacteria such as fighting the rapid bacterial growth on the effluent or any media will be addressed in the analysis section. In terms of the prototype, a design was selected to fulfill the design criteria while still stimulating what would happen in the manufacturing process particularly in the factory's water treatment unit.

In this paper, various topics will be discussed, including the materials used, the methods we did to complete our project, scientific discussion of the project, and finally, some recommendations for better research in the future.

MATERIALS AND METHODS

Firstly: Materials

Table 1: materials

Name	Photo	Cost
Bacillus subtilis slants		600 L.E for any number ofcopies
Glass container		100 L.E
Agar		21 L.E / 50 gram

Secondly: Methods

A glass container was designed with dimensions 0.5 m, 0.3m and 0.2m for length, width, and height, respectively. Having approximately 0.015% of the actual container's volume, our container has a volume of 200 cm3. From the

above of the container, there is a pipe for the water entry. In addition to the pipe, there is a faucet at the bottom for the exit of water. Below, there are steps done

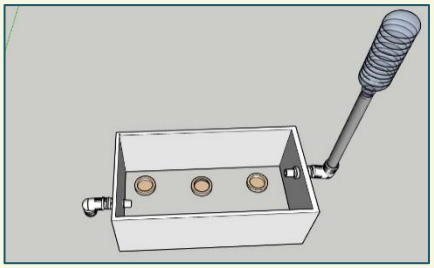


Figure 4: prototype ،Figure 3D design

- Bacillus Subtilis bacteria were cultured on regular pieces of agar.
- Agar pieces were attached to the bottom of the container.
- Then, the wastewater was put in the bottom of the container and left to interact with the bacteria.

For the test plan, it was performed through three major steps:

The amount of urea in the wastewater was measured – to ensure that the present amount of urea is suitable for the bacterial growth of B.S. using urease Berthelot method.

-Thus, the Urea Hydrolysis Reaction can initiate.

- An amount of wastewater was prepared in an Agar plate having a concentration of 2% of the agar powder then a zigzag-shaped of the B.S. at the top of the plate.
  - In order to observe the change in solution's pH, four drops of pH color indicator "phenol red" were also put in the solution.

Note that: phenol red has a pH range from 6.8 to 8.2, yellow at acidic conditions, and pink or red at alkaline conditions

- pH and COD values were measured using a piece of the processed agar plate.
  - The Agar plate was placed in deionized water for an hour. After that, pH was measured using the pH meter, and COD was measured using the COD Analysis Test.

RESULTS

Parameter	Before	After (7 days)
Urea test	5 gram/100 ml	…….
pH	6.13	7.60
COD	13614 ppm	1217 ppm

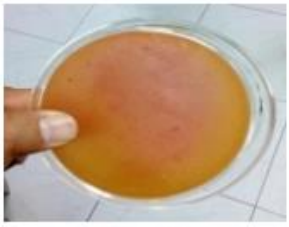


Figure 5
Red color zone appeared around inoculation line "Zigzag shape" of BacillusSubtilis (2 days)

Figure 6
pH Reading

Figure 7
Red color appeared and spread overall the surface (7 days)

ANALYSIS AND DISCUSSION

After the test plan findings have been recorded, thoughts have been presented about how the actual results should be used to address the main problems that threaten the nation's growth and development and society.

The suggested solution has addressed two big problems that constitute a genuine threat for Egypt: water scarcity and pollution. As a result of wastewater reuse in production lines, water has been reassigned, and water consumption substantially reduced. Moreover, the amount of contamination caused by the prevention of pollution has been reduced to discharge and leak into the soil or allowed to evaporate afterward to cause acidic rain, thereby causing health problems to people living nearby. Apart from this, the proposed solution has been able to increase the industrial base in Egypt. Egypt has always depended on the conventional machine industry and imports from several other countries, representing a considerable capital loss for any businessman who hopes to figure out their productive industry money. This was solved in our solution, where the bacterial water system was used to minimize the COD level, raise the pH level, and finally, make the water reusable again. The project was done based on the wastewater from the "First Paper Obour City" factory. Still, it can be said that the presented solution can be applied to other factories working with the same mechanism.

Now, we will discuss the urease enzyme "our main element to carry the reaction," pH processing, COD processing, and the B.S. bacteria environmental implications respectively after that we will draw our final conclusion.

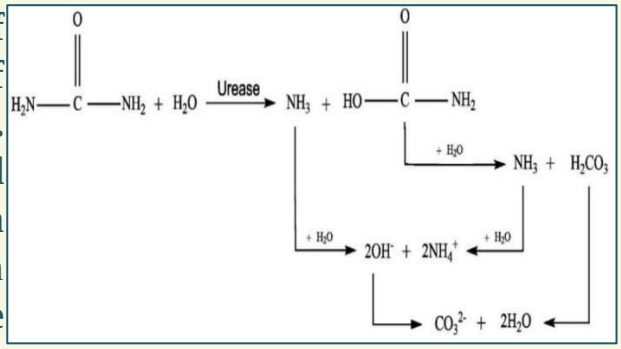
The urease enzyme

The urease enzyme is produced by the ureABC operon in the B.S. bacteria.

The ureABC operon consists of three genes respectively "ureA, ureB, and ureC"; together are responsible for the formation of the urease's three subunits, which results in the urease enzyme.

pH

As known, the basic rule is that the pH of a solution increases when the percentage of OH^- increases or the H^+ decreases. According to the results, it can be observed that there is a massive percentage of urea in the wastewater. The urease "as shown in scheme 1" served as an enzyme to carry the urea hydrolysis reaction.



Scheme 1: urea hydrolysis reaction

The pH rise occurs because ammonia, resulted from the hydrolysis reaction, has a free pair of electrons above the nitrogen atom, as illustrated in figure 9. This pair of electrons will accept an H+ ion from the water, which will raise the pH.

In addition, this reaction may take another pathway to raise the pH. The ammonia molecule can react with the water molecule forming an ammonium molecule NH4+ an OH- ion, as shown in scheme 1.

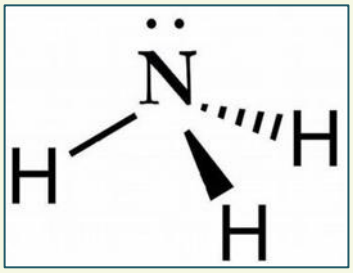


Figure 9: ammonia molecule

From the previous, it can be concluded that the ammonia molecules, resulted from the urea hydrolysis reaction, caused a H+ uptake from the effluent, which raised the pH as the ammonia is considered a Bronsted-Lowrey base.

COD

The COD of the wastewater was lowered from 13614 ppm to 1217 ppm, which means a 91% removal of the pollutants. The COD level was enhanced because the B.S. bacteria is a heterotrophic bacterium, which means that it consumes the media's chemicals as an oxygen and nutrients source for its metabolic pathways.

Environmental implications

The BS-bacteria are a nonpathogenic strain of the bacillus species. It is already found naturally in human beings, Ruminants, "herbivorous mammals," and animals mainly in the gastrointestinal tract, and it does not pose any threat on them. It is also found in grass commonly and helps the

plants in many ways. It secretes some compounds such as exopolysaccharides and siderophores that prevent the movement of the toxic ions. It metabolizes "digest" phosphates, nitrates, and plant nutrients into more bioavailable compounds for the plants.

Furthermore, it inhibits the growth of the bacterial pathogen colonies. The B.S. bacteria also has a “Bacteriocinogenic plasmids,” which is capable for producingbacteriocins that kills most of the near pathogenic colonies. Bacteriocins

from B.S. bacteria include the lanthionine-containing peptide antibiotic (lantibiotic peptide) called subtilin and an antibiotic called subtilosin. Subtilosin has proven antimicrobial activity against Gram-negative and Gram-positive bacteria as well as anaerobic and aerobic microorganisms. It is particularly effective against several human pathogens. So, we can conclude that the Bacillus subtilis bacteria has no negative environmental impact, but it has positive impacts. And this our second design requirement.

CONCLUSIONS

The data obtained, the test results, and the analysis have concluded that virtually all the required criteria have been satisfied by this bioremediation process. It has reached the standard productivity needed to launch a new or even low-cost water reuse system. As we reached the acceptable water criteria used in the paper recycling industry and other Egyptian industries, its cost is considered low with respect to Quality. Our efficiency's final parametersare similar to or near pure water parameters relatively to the untreated water, which are 7.60 pH and 1217 ppm COD. This exactly fits our water quality parameters. Furthermore, BacillusSubtilis that can live in extremely harsh conditions are used to obtain our project aims without any harm “eco-friendly” and relatively low cost.

With varying attributes, the approach presented was superior to the previous projects. Our project has handled all toxins with no negative environmental impacts and very low cost. Finally, saving millions of Egyptian Pounds associated with the water treatment units of the factory.

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RECOMMENDATIONS:

After our efforts to get a good solution and an excellent way to implement it, we recommend the researchers who will further work on our solution to consider those ideas to get higher quality:

- For maximum benefits and results, optimize the conditions for the Bacillus Subtilis growth.
- Use a urea-containing media while preparing the Bacillus Subtilis bacteria because the urea will act as an inducer for the urease enzyme production and makes the genes associated with the urease production "ureABC operon" already activated, for a faster and better results.
- We have created an agar plate of the effluent covered by a few drops of 20g soil and 100ml of distilled water mixture. So, by screening this plate containing the bacterial colonies from the soil, we may find better options than the Bacillus subtilis bacteria since we observed that some colonies could grow on the effluent "plate" and made a remarkable positive change in the water's pH, "the color of the plate became red."
- To further enhance the process in the large scale, the biofilm technology can be used to fully purify the water from the COD "pollutants."

Abbreviations and crucial definitions:

Ppm	Parts per million, equivalent to "milligram / liter"
COD	Chemical oxygen demand, which is an indicator to the level of the contamination presented in the water.
Bioremediation	Bioremediation is a process that uses micro-organisms to reduce contamination through the biological degradation of pollutants into non-toxic substances.

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