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Estimation of Bacterial Treatment Effect on Lignocellulosic Biomass in Anaerobic digester

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الملخص العربى
أصبحت تقنية الهضم اللاهوائى باستخدام الكائنات الدقيقة اللاهوائية من التقنيات الواعدة المنتشرة مؤخرا لاستخدامها فى التخلص من المخلفات العضوية بطريقة آمنة على البيئة و الاستفادة من مخرجاتها فى إنتاج الغاز الحيوى و استخدام الناتج من المواد الصلبة فى تسميد الأراضى لاحتوائها على مواد عضوية لازمة لتحسين التربة. تهدف هذه الدراسة إلى تحسين عملية الهضم اللاهوائى عن طريق رفع كفاءة مرحلة التحلل أثناء معالجة مخلفات الذرة الملقحة بالحمأة النشطة بإضافة بكتريا نشطة على مرحلتين: مرحلة المعالجة الحيوية قبل بدأ التفاعل ب 5 ايام ثم مرحلة الازدياد الحيوى مع بدأ التفاعل فى درجتين حرارة مختلفتين 37 و 55 °مئوية مع وجود مفاعل بدون معالجة لكل درجة حرارة. نتج عن ذلك زيادة فى إنتاج الغاز الحيوى بنسبة 27% تحت درجة حرارة 37° مئوية ، لكن تحت درجة حرارة 55° مئوية أعطت نتيجة سلبية نظرا لتأثر البكتريا المنتجة للميثان بدرجة الحرارة العالية و قل إنتاج الغاز بنسبة 35.7%.

Abstract:

The effect of bacterial treatment of corn waste anaerobic digestion on biogas production was studied. The bacterial treatment was applied twice; before the beginning of the reaction and with the start of reaction. The results showed that under 37°C temperature, the produced biogas increased by 27% in case of bacterial treatment with *Pseudomonas aerginosa* and *staphylococcus aureus*. Nevertheless, under 55°C temperature, the biogas production decreased by 35.7% in case of bacterial treatment because the higher temperature led to microorganism destruction.

Key Words

Anaerobic digestion, Corn waste, Biogas, Bacterial treatment.

Introduction

Anaerobic digestion (AD) process has become one of the most promising technologies that is used to turn the organic wastes into biogas(Khalid, Arshad, Anjum, Mahmood, & Dawson, 2011). This produced biogas is rich in methane about 50 to 75% that is used as alternative to natural gas to be used in power generations. Another benefit of solid waste anaerobic digestion is producing effluent rich in organics and nutrients as a natural alternative solution of fertilizers that enhance soil quality.

It is found that hydrolysis stage is the main step in anaerobic digestion of substrates with high solids content like agricultural waste.(Mumme, Linke, & Tölle, 2010). Hydrolysis is the first step of anaerobic digestion of lignocellulosic biomass in a series of anaerobic degradation phases by different groups of microorganisms. During hydrolysis, the microorganisms convert the complex substances to less complex compounds in order to facilitate the fermentation of produced monomers in the next stage. Then during acidogenesis phase, the acid forming group of bacteria convert the products of hydrolysis into less organic acids besides hydrogen and carbon dioxide. In the third stage acetogenic bacteria convert the fatty acids to acetic acid, carbon dioxide and hydrogen. Finally, the methanogenic bacteria convert the acetic acid and the remaining hydrogen into methane and more of carbon dioxide(Parkin & Owen, 1986).

Applying the AD in solid state (TS>15%) enhance the lignocellulosic biomass hydrolysis step in order to increase the efficiency of the digestion process and raise the economic viability of the digester in terms of methane production, fertilizing digestate(Martin, Potts, & Heslop, 2003). There are different methods to enhance fermentation of lignocellulosic biomass, including the chemical, physical or biological pretreatment. Biological treatments are a processes of adding specific strains of microorganisms that work on degradation of complex carbohydrates existed in the substrate to improve its digestibility(Schauer-Gimenez, Zitomer, Maki, & Struble, 2010).

Therefore, this research will investigate the effect of biological treatment with specific strains of bacteria on the solid state anaerobic digestion (SS-AD) of corn waste in terms of (biogas production, substrate hydrolysis rate and solids reduction) under mesophilic and thermophilic temperatures.

Materials and Methods

Materials

The used corn waste was brought from a field in Menofia, Egypt. The waste was dried and milled using a kitchen machine. The grinded waste was elementally and chemically analyzed with the Standard Methods for the Examination of Water and Wastewater(APHA, 2005) in the water quality lab of the faculty. Values of Total solids (TS), volatile solids (VS), volatile fatty acids (VFAs), carbohydrates, lipids and C/N ratio are listed in Table 1.

The inoculum used in this research was sludge collected from Abu-Rawash wastewater treatment plant, Giza city, Egypt. The used microorganisms were prepared in microbiological laboratory of Housing and Building National Research Center (HBRC). Isolated bacterial strains were *Pseudomonas aerginosa* and *staphylococcus aureus*.

Table 1: Characteristics of collected corn samples

Corn Characteristics	
Test	Corn sample
Total solids (%)	95.4
Total volatile solids (%)	85.8
Volatile fatty acid (%)	2.42
Lipids (%)	0.68
Carbohydrates (%)	74.6
Total carbon (%)	43.8
Total Nitrogen (%)	1.23

Experimental work

The experiments were conducted using four flasks of 2L volume as batch reactor in microbiology lab of HBRC. The flasks were tightly sealed using rubber stoppers to keep the anaerobic condition, connected to gas syringe to measure the produced biogas volume. They were divided to 2 groups: (R1) Non-treated reactors, (R2) Treated reactors. Before the digestion process beginning, two reactors (R2) were loaded with 50 g of dry grinded corn waste then they were pretreated with the prepared bacterial strains (*P. aerginosa and S. aureus*), tightly sealed with rubber stopper then they were kept in room temperature (35)°C for 5 days.

All reactors contained a mixture of 50 g of dried corn waste, 50 ml of distilled water with 200 ml of fresh sludge in order to keep the TS approximately higher than 15% to maintain SS-AD condition at the reaction beginning. Only microbiological pretreated reactors (R2) were bioaugmented again with the isolated bacterial strains with 2% (v/v) inoculum size for each. The batch reactors were kept at incubation temperatures 37 and 55°C during the AD retention time (35 days) using lab incubators with intermittent shaking (100 rpm). Samples were collected every 5 days for the analysis of total solids (TS), total organic carbon (TOC) and total nitrogen (TN) to obtain C/N ratio in addition to measuring PH value. A sample of gas was analyzed to recognize the produced biogas.

Analytical Methods

Solids analysis (TS) determination were applied using standard methods for the examination of water and wastewater(APHA, 2005) in the microbiology lab at HBRC. Walkley-Black titration method was followed to determine TOC and analysis of TN were determined using micro Kjeldahl method(A.O.A.C., 1995). PH meter was used to determine PH value. Biogas samples were analyzed using natural gas analyzer with TCD detector.

Results and Discussion

TS behavior

Values of TS were recorded during the anaerobic digestion process every 5 days. For groups (R1 and R2), it was noticed that TS content have decreased rapidly during the start-up period, (Figure 1 &Figure *2*). This indicates the presence of high activities of microbes in the hydrolysis phase of AD where complex organic matters are being decomposed to soluble molecules by microorganisms. Higher temperature helped in increasing the rate of decrease of TS content during the hydrolysis phase which indicates that increasing temperature enhance decomposition of organic matter. At the end of AD process, the reactors achieved different values of TS reduction as follows: At 37°C, reactors R1 and R2 recorded 21.7, 29.9% TS reduction respectively; At 55°C, reactors R1and R2 recorded TS reduction 28.5, 35.7% respectively. It was noticed that with the effect of high temperature (55°C), slope of R2 get closer to R1 slope indicating that high temperatures inhibit the bacterial activity that appeared clearly in last week of the experiment time.

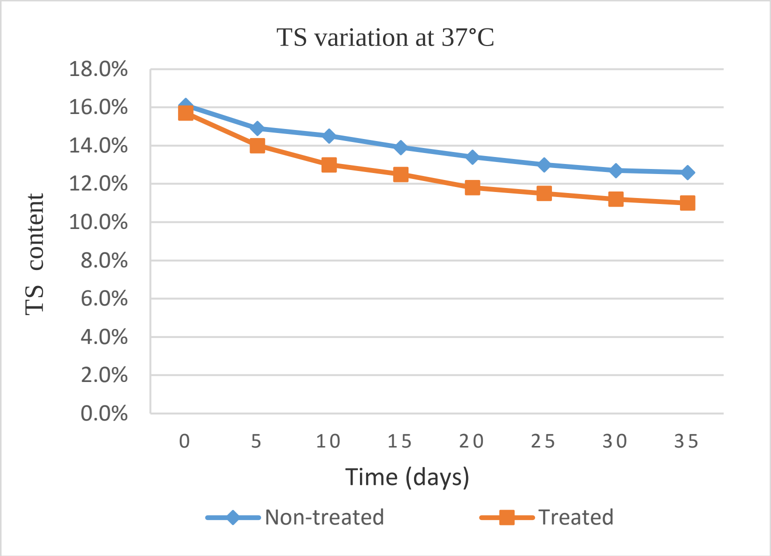

Figure 1: Total solids percentages at 37°C

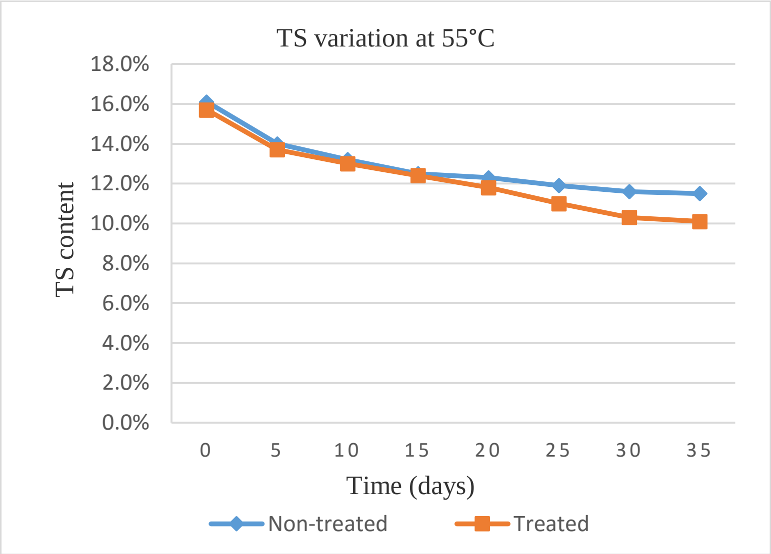

Figure 2: Total solids percentages at 55°C

PH variations

The PH values of the reactors R1 and R2 were 7.6, 7.5 at the beginning of AD process respectively. During the 1st week, PH values were decreasing slightly in group R1 then it was followed by significantly decrease till the end of 3rd week, Figure 3&Figure 4. In contrast, group R2 decreased till the end of 2nd week only. Higher microbial activity of R2 caused faster degradation of lignocellulosic substrate that led to faster generation rate of VFAs causing this decrease of PH. After the end of VFAs formation and drop of PH value, the acetic acid was consumed by some of methanogenic bacteria species that converts organic nitrogen into ammonia leading to increase of PH values till the end of AD process. The methane formation was conserved with the PH between 6.5 and 7.5. High temperature of 55°C had a negative effect on reactors R1 and R2, the PH continued to decrease less than 6.5 to the end of the experiment that inhibited the methanogenic activity.

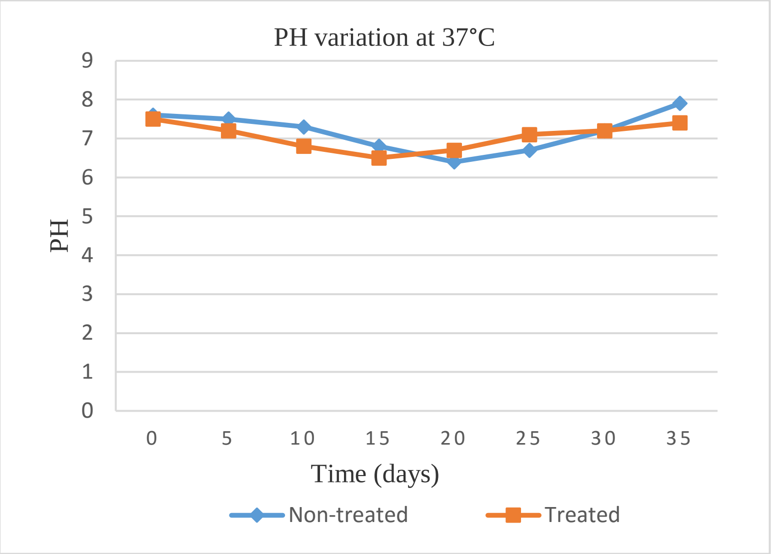

Figure 3: Changes in PH values during AD process at 37° C

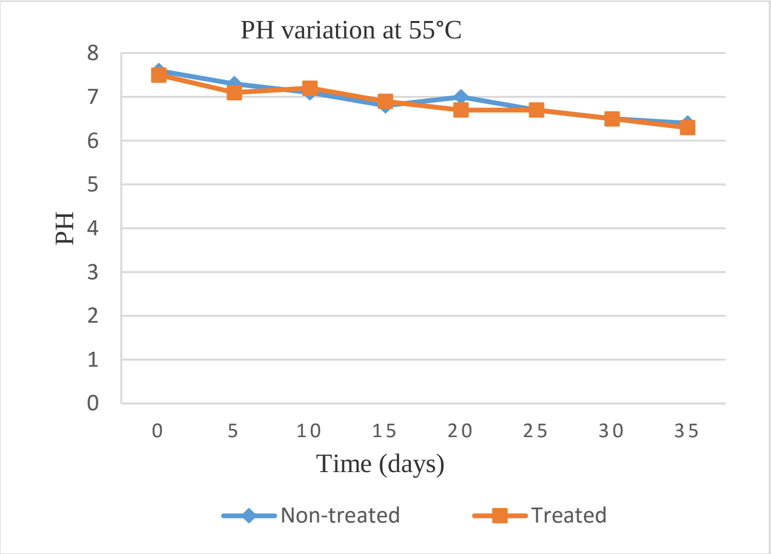

Figure 4: Changes in PH values during AD process at 55° C

Effect on C/N ratio

The values of C/N ratio of the non-treated and treated reactors started with 31.6 and 28.1 respectively. During the AD retention time, the total organic carbon of substrate is being consumed by the microbial activity in addition to consuming few amounts of nitrogen that cause decrease in C/N ratios gradually(Tchobanoglous, Theisen, & Vigil, 1993). After 35 days of digestion process the reduction of C/N ratios were as follows: at 37°C the reduction in reactors R1 and R2 were 41.8% and 51.6% respectively which indicates that the activity of bacteria enhanced the degradation of cellulose and lignin to increase biogas production, Figure 5. At 55°C the reduction were 12.3% and 18.5% respectively, this result shows that high temperature inhibited the degradation of organic matter in non-treated and also in treated with bacteria reactors, Figure 6.

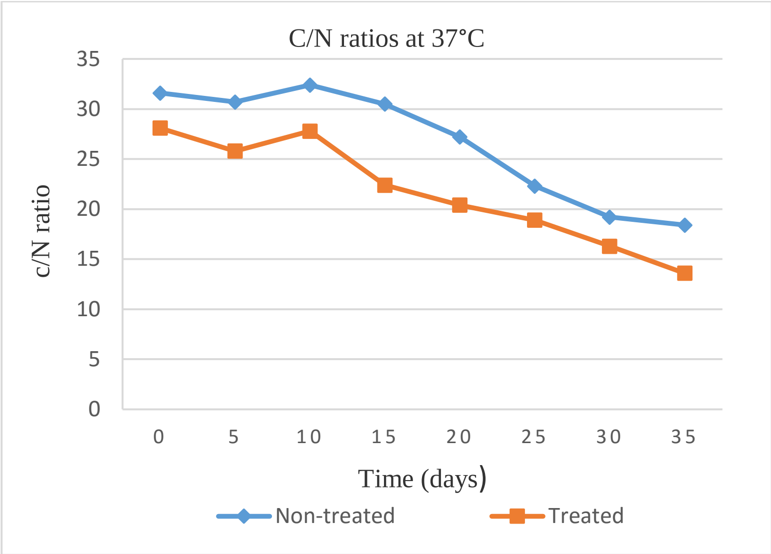

Figure 5: C/N ratio consumption during AD retention time at 37°C

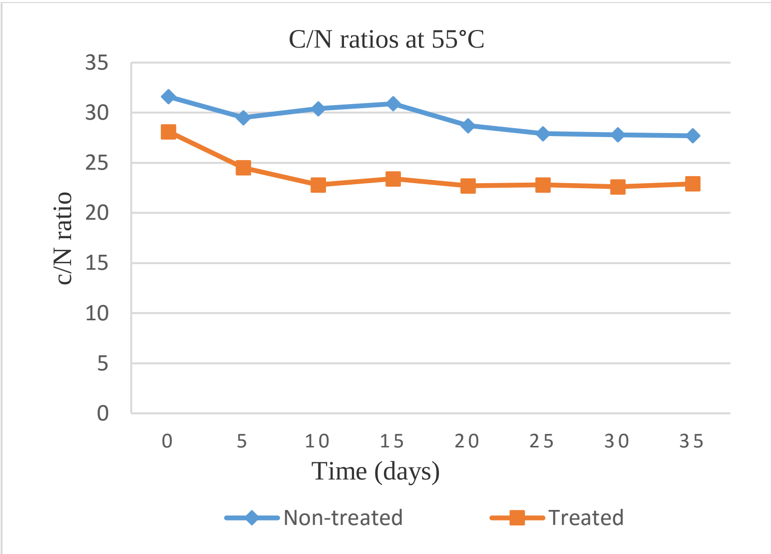

Figure 6: C/N ratio consumption during AD retention time at 55°C

Biogas production

Biogas productions of untreated (R1) and pretreated (R2) reactors were measured through the experiment retention time (35 days). The values of accumulative biogas produced from the process of anaerobic digestion are presented in Figure7 &Figure *8*. The cumulative biogas production of untreated substrate was 24.0 l/kg at mesophilic temperature 37°C that was raised up to 32.5 l/kg in case of bacterial treatment with *P. aeruginosa* and *S. aureus*.

On the other hand the value of accumulated biogas of untreated substrate was 20.5 l/kg in thermophilic conditions 55°C which gives less value than the value of mesophilic conditions. In case of bacterial addition, the accumulated biogas decreased to be 13.5 l/kg.

These results shows that microbial addition enhanced the accumulative biogas production with 27% in case of using bacteria compared to the case of no treatment under mesophilic conditions 37°C. As for higher temperature 55°C, the bacterial addition gave a negative result that decreased the accumulated biogas by -35.7%. So it can be noticed that higher temperature inhibit the microbial activity in case of no treatment and in case of bacterial addition (*P. aeruginosa and S. aureus*).

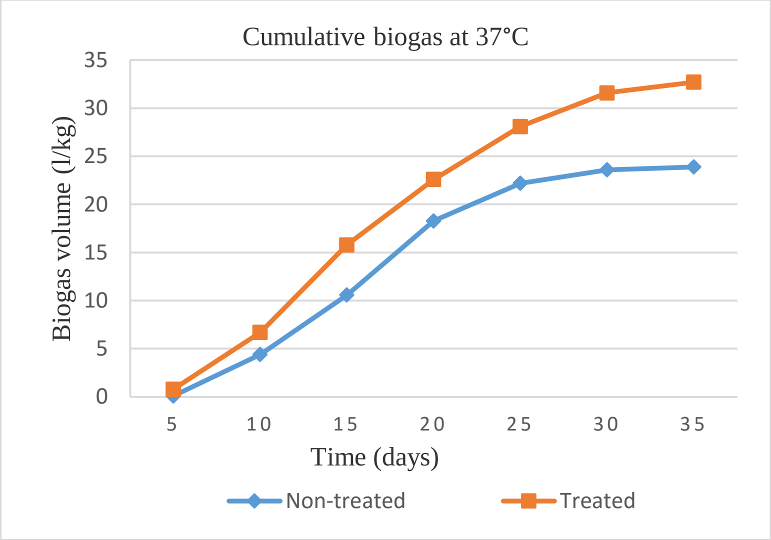

Figure7: Cumulative biogas production through AD process time at 37°C

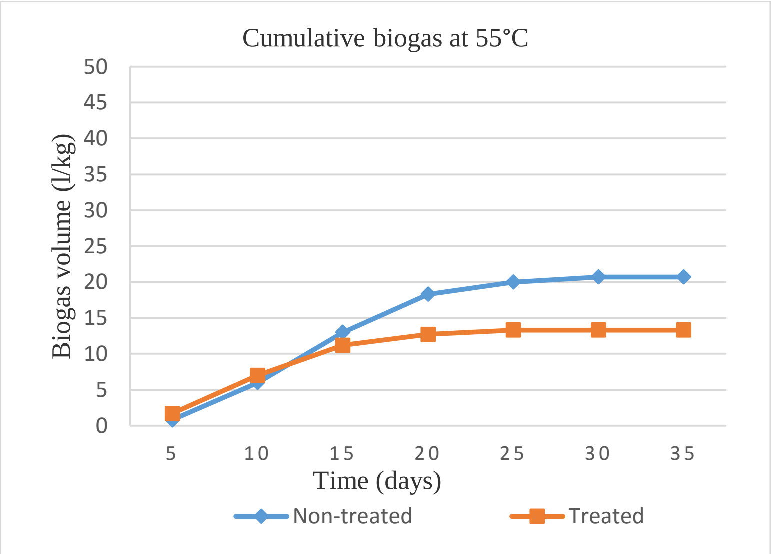

Figure 8: Cumulative biogas production through AD process time at 55°C

Conclusion

Results indicated that biological treatment in two steps was beneficial in improving anaerobic digestion of corn wastes in terms of increasing rates of substrate hydrolysis and methane production. The ability of (*Pseudomonas aerginosa* and *staphylococcus aureus*) bacteria to degrade lignocellulosic substrate efficiently and enhance its digestibility with favorable conditions of PH, C/N ratio and increase of biogas production by 27% in mesophilic condition but gave a negative result in thermophilic condition that inhibited the methane production. Methane from biogas produced from this anaerobic digestion can be used for heat or power generation. The solid effluent produced from this digestion that are rich in microbial biomass can be utilized for fertilizing products.

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