

Optimized operational parameters of anaerobic cellulosic-wastewater treatment for bioenergy recovery and effluent quality improvements

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Received: 30 Oct. 2019 / Accepted 10 Dec. 2019 / Publication date: 20 Dec. 2019

ABSTRACT

A series of standardized batch experiments were carried out to optimize the biogas production from cellulosic-rich wastewater treatment. The experimental results showed that the best result was produced under C/N ration of 20 with 80.94 % COD removal and cumulative biogas production reached 44.55 ml/g COD added. The maximum cellulose degradation was achieved at 10 g/L with a cumulative biogas production of 6.13 L/L. Based on the response surface model, the most significant operational parameter was cellulose concentration and C/N ratio with Log Worth values reached 2.112 and 1.131, respectively. As for biological pretreatment, the experimental results showed that the pretreatment under shaking cultural conditions produced better results in COD removal efficiency however, the biogas production was negatively affected as well as the soluble by-products concentrations were significantly decreased. The experimental results showed that the changing in the soluble by-product concentrations could explain the effect of operational factors and the best condition was reported.

Keywords: Anaerobic treatment, pretreatment, C/N ration, pH, Cellulose concentration, Biogas production

Introduction

Nowadays, wastewater reuse is a promising and sustainable solution especially the demand for water continues to rise as a key component of industry, agriculture and community progress, as well as high energy prices. However, wastewater reuse applications face several limitations that include public awareness, economic, health risk, and efficient treatment technology (Gadow *et al.*, 2016; Kumari & Das, 2019). The biological treatment offers several advantages to chemical and physical treatments however, the low-rate, low-startup and inhibition factors are the most drawbacks. The industry sector is considered one of the most important water consumers furthermore, the generated wastewater contained in most cases the high concentration of recalcitrant organics due to competition and innovations. For example, about 273-455 M³ is generated per ton of paper produced. The characterization of this type of wastewater is a high concentration of biochemical oxygen demand (BOD), chemical oxygen demand (COD) and color (Khanna *et al.*, 1990; Singh *et al.*, 1994). In the literature, the treatment of cellulosic-rich industrial wastewater could be classified into three different technologies. The physical treatment, the first, used different techniques such as reverse osmosis and ultrafiltration and the strong sorbent (activated carbon). As for the chemical treatment, the second, it required coagulation and precipitation process by coagulants such as alum, ferric chloride however the large of coagulants is needed that is most limitations in this treatment (Bhuptawat *et al.*, 2007). The application of the above treatment technologies still faces several limitations such as the cost and the difficulty of operating. Therefore, biological treatment including aerobic and anaerobic processes presents a promising technology (Louhichi *et al.*, 2019; Ray & Ghangrekar, 2019). From the engineering point of view, the anaerobic treatment using mixed culture is one of the sustainable and environmental processes to treat a wide range of industrial wastewater composition. On the other hand, the operational factors and stability of the treatment still need to improve as well as deal with inhibitors. Therefore, this study aimed to identify the variables significantly related to optimize the biogas production and COD removal efficiency from cellulosic wastewater treatment using anaerobic

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mixed microflora. The study also aimed to evaluate the interaction between the different operational factors using the response surface model.

Materials and Methods

Activity test experiments

The activity experiments were conducted in glass bottles (120 mL) to characterize and compare the efficiency of the mixed microflora on the recalcitrant organic-rich wastewater treatment. In each bottle, with a working volume of 80 ml, was added 5 ml of inoculum to 75 ml of synthetic medium containing (mg/l) 0.35 HBO₃, 0.5 MnCl₂·4H₂O, 0.05 ZnCl₂, 37 K₂HPO₄, 67 KH₂PO₄, 2000 NaHCO₃, 0.164 Na₂SO₃·5H₂O, 0.05 (NH₄)₆Mo₇O₂₄·4H₂O, 22 CaCl₂·2H₂O, 15 MgCl₂, 0.038 CuCl₂·2H₂O, 5 FeCl₃·6H₂O, 0.09 NiCl₂·6H₂O, 0.09 AlCl₃·6H₂O, 11 CoCl₂·6H₂O and 40 ml reducing solution (200 mL NaOH (0.2 M); 2.5 g Na₂S·9H₂O; 2.5 g cysteine HCl·H₂O). According to the surface response model, three different concentration levels (10, 55 and 100 g/l) of cellulose were used to investigate C/N ratio effects. As for the pH, four different levels of 6, 6.61, 7 and 8 were selected. The pH was adjusted upward with 10% NaOH or decreased with 6 N HCl. Once the pH had stabilized (at least 5 min), the medium containing anaerobic mixed microorganisms was incubated. All treatments were incubated under mesophilic temperature at 30±2°C. Besides the test bottles, in which both inoculum and synthetic medium were contained, a control bottle of inoculum employed. The bottles were incubated in the shaking with temperature control.

Biological pre-treatment

Two different batch experiment series was conducted to investigate the effect of aerobic hydrolysis of recalcitrant organic-rich wastewater on bioenergy recovery and COD degradation efficiency. The batch experiments were inoculated with activated sludge under static and shaking culture conditions at 30±2°C. The incubation period was 6 days and then all treatments were anaerobically incubated for biogas recovery and COD removal. The reducing sugar and amylase were determined in the treatments before biogas production.

Source of microorganisms

The anaerobic mixed microflora was collected from Gabal El-Asfar Wastewater Treatment Plant (GAWWTP), Madinet Al Khankah, Al Khanka, Al Qalyubia Governorate. The anaerobic inoculum was passed through a 2 mm sieve and kept at a mesophilic temperature (30±2°C).

Analytical methods

The volatile suspended solid (VSS), total suspended solid (TSS), volatile solid (VS) and total solid (TS) were measured according to the procedures described in the Standard Methods (Rice *et al.*, 2012). The COD was measured by semi-automated colorimetry. Sample, blanks, and standards in sealed tubes are heated in block digester in the presence of dichromate at 150°C. After two hours, the tubes are removed from the oven or digester, cooled, and measured spectrophotometrically at 600 nm. The colorimetric determination may also be performed manually.

Results

Effect of C/N ratio on the optimization of bioenergy production

A series of activity test experiments were conducted to investigate the effect of the C/N ratios on biogas yield and COD degradation (see fig.1). Three different levels of C/N ratios of 20, 40 and 60 were investigated to assess the impact on the treatment performance. The experimental results showed that the best result was produced under C/N ration of 20 with 80.94 % COD degradation and cumulative biogas production reached 44.55 ml/g COD added. The results showed an inverse relationship between nitrogen concentration and degradation efficiency and/or energy recovery efficiency.

Table 1: Effect of C/N ratio on mixed microflora performance on the optimization of bioenergy production.

C/N ratio	Cumulative biogas production L/L	Biogas ml/g COD _{add}	Biogas ml/g COD _{rem}	SCOD g/L	TCOD g/L	COD degradation %
20.00	6.13±0.14	44.55	55.04	1.51	2.10	80.94
40.00	3.96±0.08	28.82	42.73	2.66	3.58	67.45
60.00	3.08±0.11	22.36	39.01	1.44	4.69	57.33

At C/N ratio of 60, the biogas yield was decreased by 49.8 % compared with the optimal C/N ratio recorded. Another important finding is that the biogas conversion and soluble by-products concentration was improved at a low C/N ratio with maximum bioconversion reached 55.04 ml biogas per gram COD removal (see table 1).

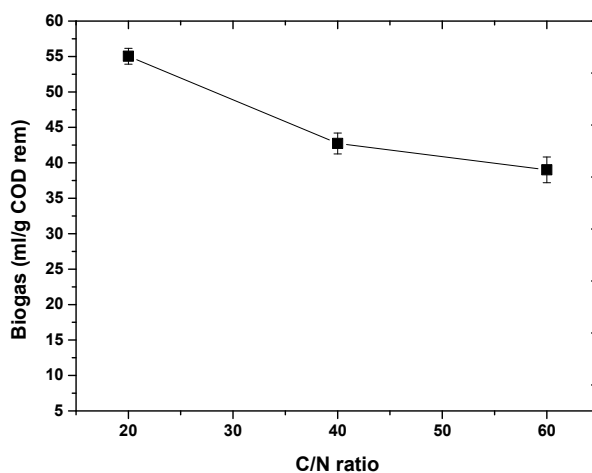


Fig. 1: The effect of C/N ratio on biogas production

Effect of pH on biogas production and COD removal

The effect of pH on the anaerobic mixed bacteria activity and changing in the soluble COD concentrations was investigated. Four different pH levels were studied during cellulosic wastewater treatment and bioenergy conversion (see fig. 2). The experimental results showed that the maximum activity and biogas yield were recorded at pH of 8. On the other hand, the lowest results were obtained at pH of 6.

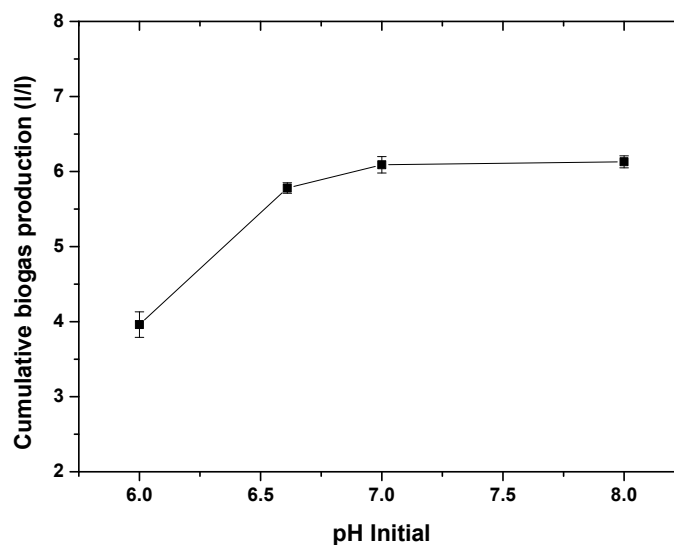


Fig. 2: The effect of pH on the anaerobic mixed bacteria activity

Table 2: Effect of pH on mixed microflora performance on the optimization of bioenergy production.

pH	Cumulative biogas production L/L	Biogas ml/g COD _{add}	Biogas ml/g COD _{rem}	SCOD g/L	TCOD g/L	COD degradation %
6.00	3.96±0.08	28.82	42.73	2.66	3.58	67.45
6.61	5.78±0.13	4.57	14.58	4.00	69.31	31.37
7.00	6.09±0.16	8.70	27.01	3.55	37.97	32.20
8.00	6.13±0.14	44.55	55.04	1.51	2.10	80.94

Effect of cellulose concentration on the COD degradation efficiency

Recalcitrant organics containing wastewater such as cellulosic wastewater is always considered a big challenge for scientific communities because of socio-economic factors. Therefore, the effect of different cellulose concentrations on COD degradation and effluent quality was investigated. Three different concentrations of cellulose (10, 55 and 100 g/l) were examined using a controlled batch activity test (see fig. 3). The experimental results showed that an inverse relationship between cellulose concentration and degradation efficiency and/or energy recovery efficiency was observed (see table 3). The maximum cellulose degradation was achieved at 10 g/l with a cumulative biogas production 6.13 l/l. Another important finding was that there is no significant difference between the soluble by-products concentrations under 55 and 100 g/l.

Table 3: Effect of cellulose concentration on COD degradation and effluent quality.

Cellulose conc. g/l	Cumulative biogas production L/L	Biogas ml/g COD _{add}	Biogas ml/g COD _{rem}	SCOD g/L	TCOD g/L	COD degradation %
10.00	6.13±0.14	44.55	55.04	1.51	2.10	80.94
55.00	8.34±0.18	11.91	26.68	5.63	31.00	44.64
100.00	8.73±0.24	6.91	17.33	5.07	70.72	29.88

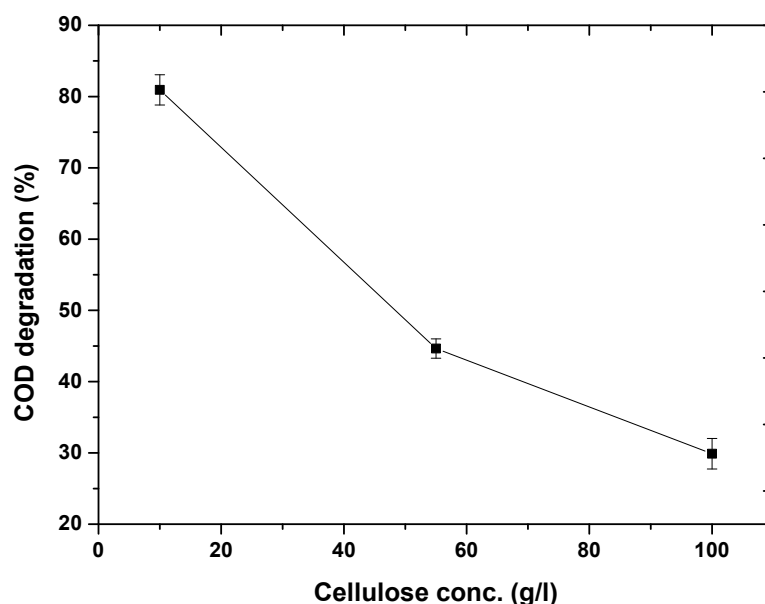


Fig. 3: The effect of cellulose concentration on COD degradation in the anaerobic cellulosic wastewater treatment

Response surface model of the interaction between different operational parameters

Based on the response surface model, the interaction between C/N ratio, pH and cellulose concentration was studied. Table 5 shows that the most significant operational parameter was cellulose concentration, C/N ratio and their interactions with LogWorth values reached 2.112, 1.131 and 1.092, respectively. On the other hand, the pH and its interactions with C/N ratio and cellulose concentrations showed no significant changes in the COD removal and biogas yield optimization. Based on the results obtained, the biogas production ranged from 3.15 to 44.55 ml/g COD added.

Furthermore, the maximum accumulative soluble by-products were recorded at C/N ratio of 60 and 55-gram cellulose per liter. Although there is an improvement in biogas production by increasing cellulose degradation, it was found that the biogas production and total COD added ratio was decreased (see table 4). The simulation of the predicted performance using the response surface model showed that the value of the root mean square error (RMSE) was higher at cumulative biogas production and COD degradation (see figure 4). Furthermore, the PValue of cumulative biogas production predicted reached 0.2476 with 0.98 R².

Table 4: The interaction between different operational parameters and LogWorth values

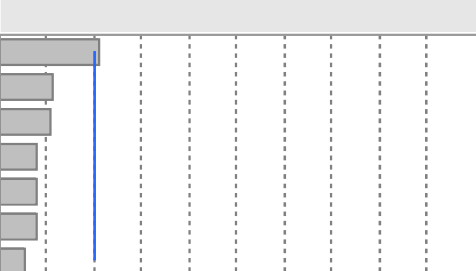
Source	LogWorth		PValue
Cellulose conc. g/l	2.112		0.00773
C/N ratio	1.131		0.07388
Cellulose conc. g/l*C/N ratio	1.092		0.08098
pH*C/N ratio	0.804		0.15722
pH	0.794		0.16065 ^
pH*Cellulose conc. g/l	0.783		0.16468
pH*Cellulose conc. g/l*C/N ratio	0.562		0.27403

Figure 5 shows the overall performances and it can be noted that there is a linear relationship between soluble by-products concentration and the operational parameters however, other performance showed an inverse relationship. It can also be concluded that the changing in the soluble by-products concentration explained the effect of operational factor and the best operational condition was evaluated.

Effect of pretreatment on the biogas production and COD removal

A series of standardized activity experiments were carried out to study the influence of two different biological pretreatments on biogas improvement and COD removal efficiency from cellulosic wastewater treatment. Under static cultural conditions, the maximum COD removal was reached 85.82% with 1.71 L/L cumulative biogas production. Generally, the biological pretreatment using static cultural condition produced a better result in COD degradation efficiency however, the biogas production was negatively affected as well as the soluble by-products concentrations were significantly decreased (see Table 5). The Effect of different biological pretreatment conditions on cumulative biogas production and COD degradation efficiency using different cellulose concentration and C/N ratios was presented in figures 6, 7, and 8. At 10 g/l cellulose and C/N ratio of 20, even though the cellulose degradation was improved significantly under shaking condition, the accumulative biogas production was extensively decreased by 81.2 %. On the other hand, under C/N ratio of 40, there is no significant change in the accumulative biogas production while the effluent was improved significantly.

Our results reported that the biological pretreatment under shaking conditions improved the COD removal efficiency and accumulative biogas production under high C/N ration. As for 55 g/L cellulose concentration, both biological pretreatment conditions negatively affected accumulative biogas production however, the COD removal was enhanced with 86.37 % under C/N ratio of 60. In the same line, at 100 g/l cellulose concentration, the shaking and static culture conditions decreased the biogas production under 20 and 40 C/N ratios, however, it was improved significantly under C/N ratio of 60. Our results reported that the culture condition is a key factor to optimize the cellulosic rich wastewater with high C/N ratios.

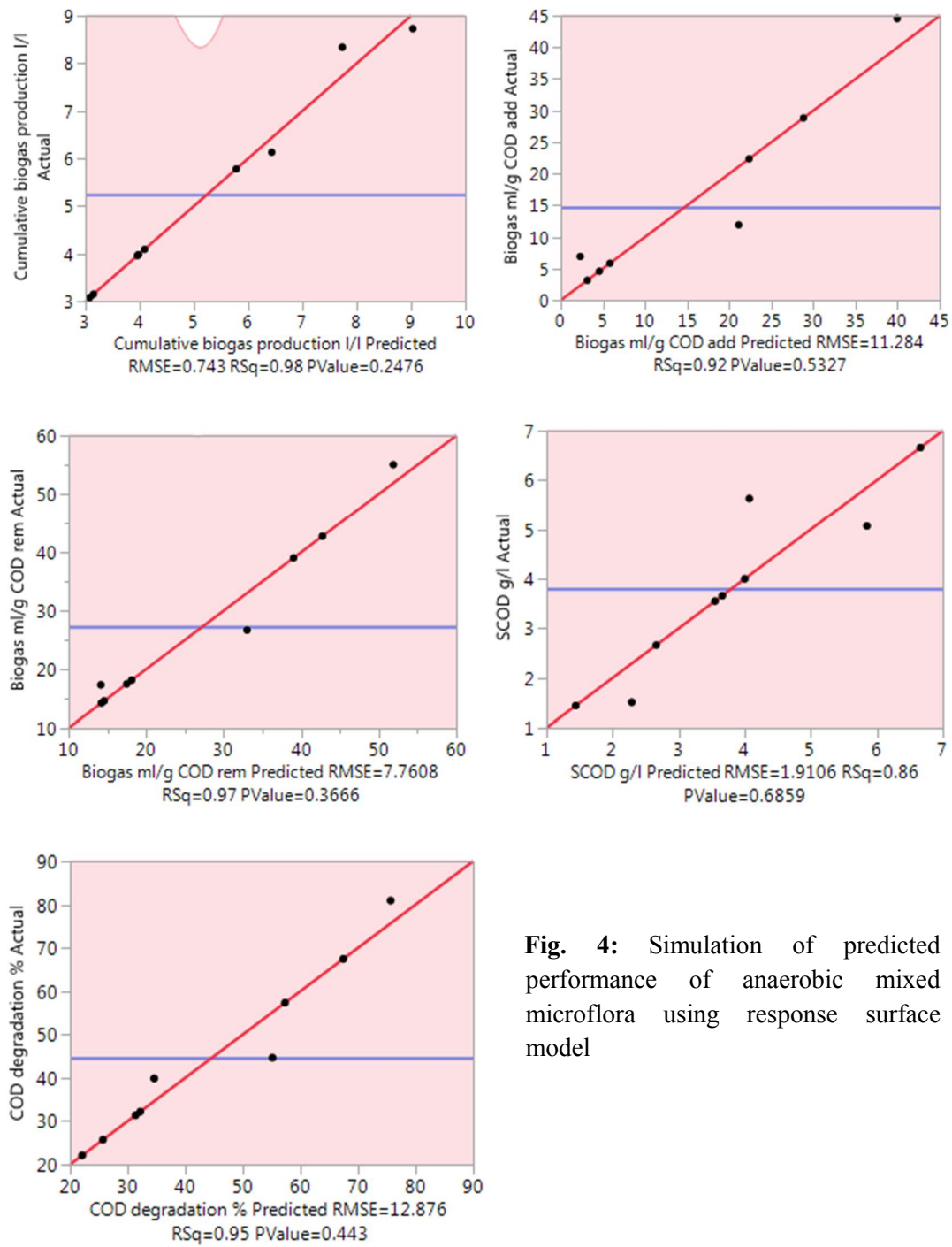


Fig. 4: Simulation of predicted performance of anaerobic mixed microflora using response surface model

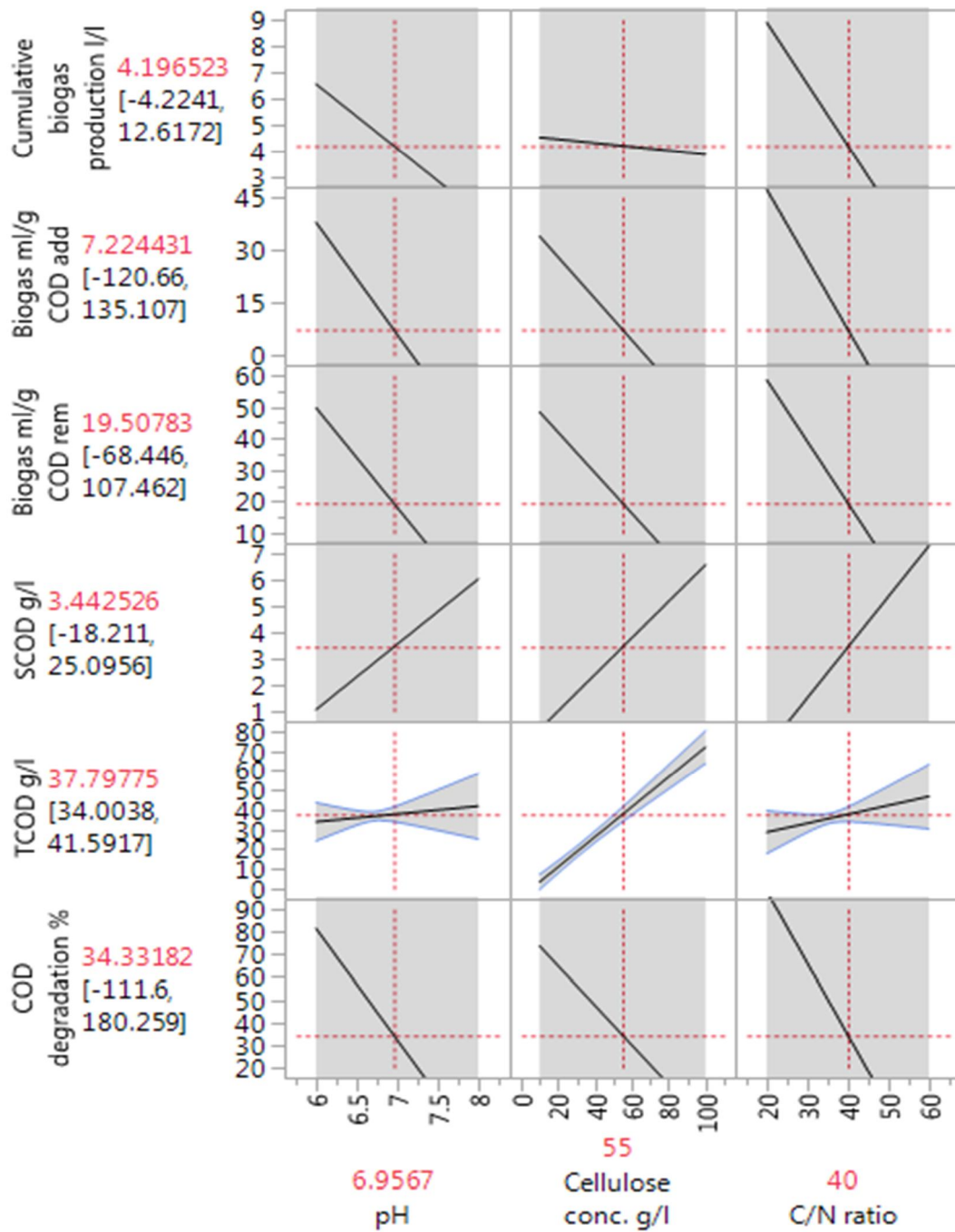


Fig. 5: The effect of pH, Cellulose concentration and C/N ratio on anaerobic mixed cellulose microflora

Table 5: Effect of pretreatment on the biogas production and COD removal

Treatment	Cumulative biogas production L/L	Biogas ml/g COD ^{add}	Biogas ml/g COD ^{rem}	SCO D g/L	TCO D g/L	COD degradation %
Biological pretreatment static cultural conditions						
T10	1.63±0.03	5.9	7.5	1.62	2.30	79.09
T11	0.94±0.04	6.8	8.2	0.87	1.88	82.91
T12	1.71±0.03	12.5	14.5	1.13	1.56	85.82
T13	0.96±0.01	1.4	1.7	2.31	10.5	81.25
T14	1.65±0.04	2.4	2.8	2.44	12.66	77.39
T15	2.20±0.03	3.1	3.8	2.71	9.84	82.43
T16	0.73±0.02	0.6	1.8	1.57	68.3	32.38
T17	1.50±0.08	1.2	3.1	3.14	62.27	38.35
T18	0.48±0.06	0.4	0.8	2.88	53.22	47.31
Biological pretreatment shaking cultural conditions						
T19	1.15±0.04	8.4	12.9	0.87	1.28	88.36
T20	4.06±0.12	29.5	37.4	2.32	1.71	84.45
T21	3.99±0.10	29.0	35.4	1.22	1.98	82.00
T22	1.03±0.14	1.5	1.7	1.32	7.89	85.91
T23	0.00±0.00	0.0	0.0	0.81	8.96	84.00
T24	0.05±0.00	0.1	0.1	0.74	7.63	86.37
T25	0.66±0.01	0.5	1.1	1.88	53.75	46.78
T26	0.83±0.08	0.7	1.4	3.31	53.84	46.69
T27	5.45±0.11	4.3	7.5	5.44	42.61	57.81

Discussion

Optimization of biogas production and cellulose degradation

A large quantity of cellulosic wastewater produced by industrial activities such as paper industries that are highly polluted organically. The treatment of cellulosic wastewater by anaerobic methods offers several focal points, such as the elimination of contamination and the avoidance of odor leakage (Gadow *et al.*, 2016; Jiang *et al.*, 2018). Therefore, a twenty-seven of anaerobic standardized activity experiments were carried out to optimize the biogas production and recalcitrant organic-rich wastewater treatment. The experimental results suggested that the effect of operational parameters such as C/N ratios, cellulose concentrations, pH and biological treatments could be significantly optimized for the treatment performance and cellulosic-bioenergy conversion into a safe and clean form of energy. Regards of the different attempts to biologically treatment of cellulosic wastewater, the effect of pH, C/N ratio, cellulose concentration, and biological pretreatment conditions have been reported as follows:

Effect of C/N ratio on the biogas production optimization

Three different levels of C/N ratios of 20, 40 and 60 were investigated to optimize biogas production and improve the treatment performance. The experimental results showed that the best result was produced under C/N ration of 20 with 80.94 % COD degradation and cumulative biogas production reached 44.55 ml/g COD added. On the other hand, the biogas production and COD removal were negatively affected by high C/N ratios. The above findings suggested that the microbial community structure changed due to the growth inhibition caused by low nitrogen availability in the media (DU *et al.*, 2014; Jaramillo *et al.*, 2017; Yan *et al.*, 2015). Consequently, the C/N ratio plays an important and significant role in biogas production and COD removal efficiency (Dai *et al.*, 2016; Wang *et al.*, 2014). Our results are in accordance with Fu (Fu *et al.*, 2012), a C/N ratio that is 40 will inhibit biogas production and reduce COD removal efficiency (Adekunle & Okolie, 2015; Fu *et al.*, 2012; Mao *et al.*, 2019).

Effect of pH on soluble by-product concentration and effluent quality

In this study, four different pH levels were evaluated during cellulosic wastewater treatment. The experimental results suggested that the pH of 8 produced the maximum activity and biogas yield however; the lowest results were obtained at the other pH levels. As the LogWorth decrease, the p-value is greater, meaning that the pH and its interactions with other factors were irrelevant to the

treatment improvements. Previous studies reported that the methane producing bacteria need a slightly alkaline environment from pH 6.6 to 8.5 has been reported (Omil *et al.*, 1997; Sun *et al.*, 2019). On the other hand, acid-forming bacteria grow much faster than methane forming bacteria. If acid-producing bacteria grow too fast, they may produce more acid than the methane forming bacteria can consume building up in the system. An excess of acid can drop the pH inhibiting the activity of methane forming bacteria. Also, if the pH falls below 6, anaerobic degradation rate will decrease and fatty acids will not be degraded (Dai *et al.*, 2016; Omil *et al.*, 1997).

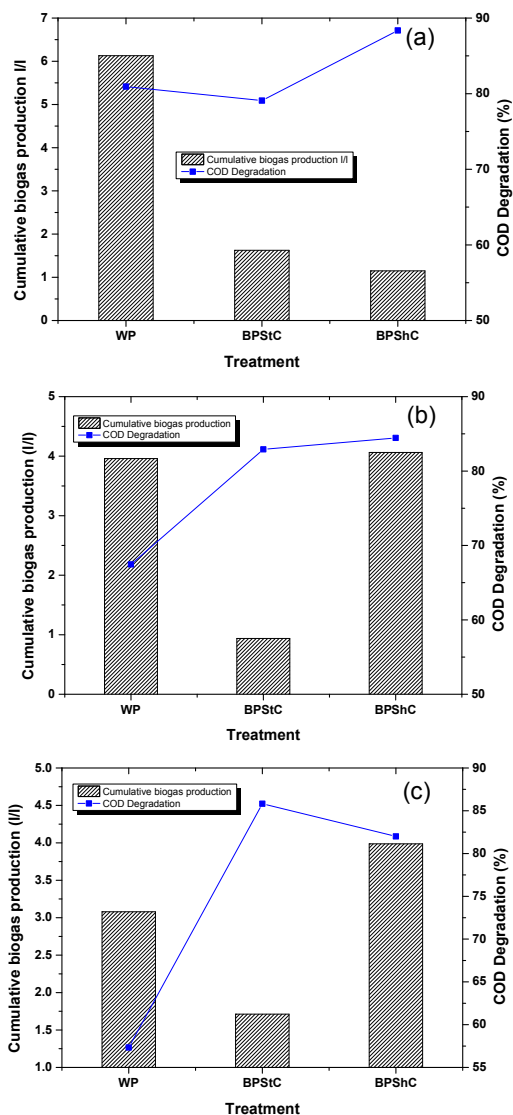


Fig. 6: Effect of different biological pretreatment conditions on a cumulative biogas production and COD degradation efficiency using cellulose concentration of 10 g/l under different C/N ratios (a) 20, (b) 40 and (c) 60.

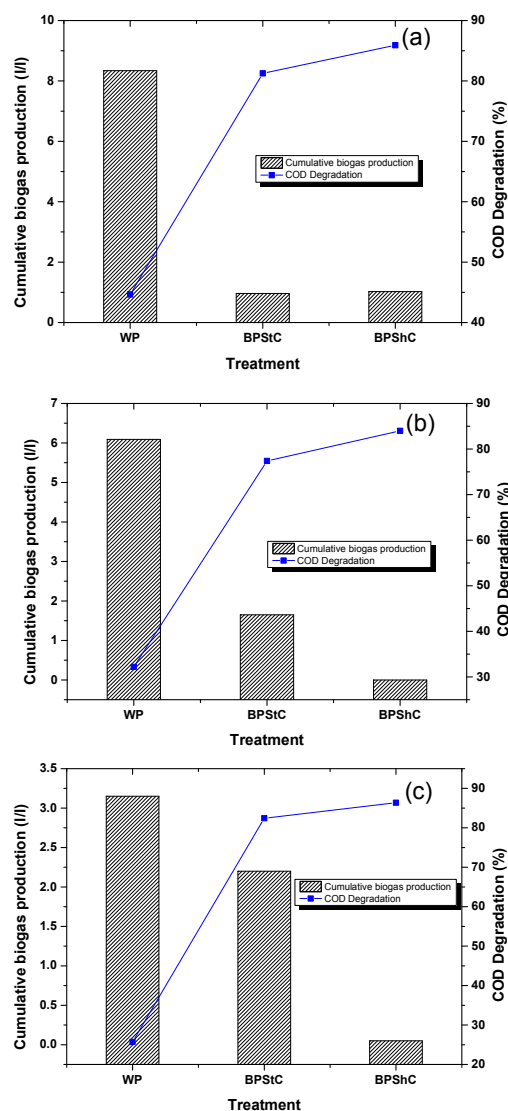


Fig. 7: Effect of different biological pretreatment conditions on a cumulative biogas production and COD degradation efficiency using cellulose concentration of 55 g/l under different C/N ratios (a) 20, (b) 40 and (c) 60.

WP, Without pretreatment; *BPSiC*, Biological pretreatment with static condition; *BPShC*, Biological pretreatment with shaking condition

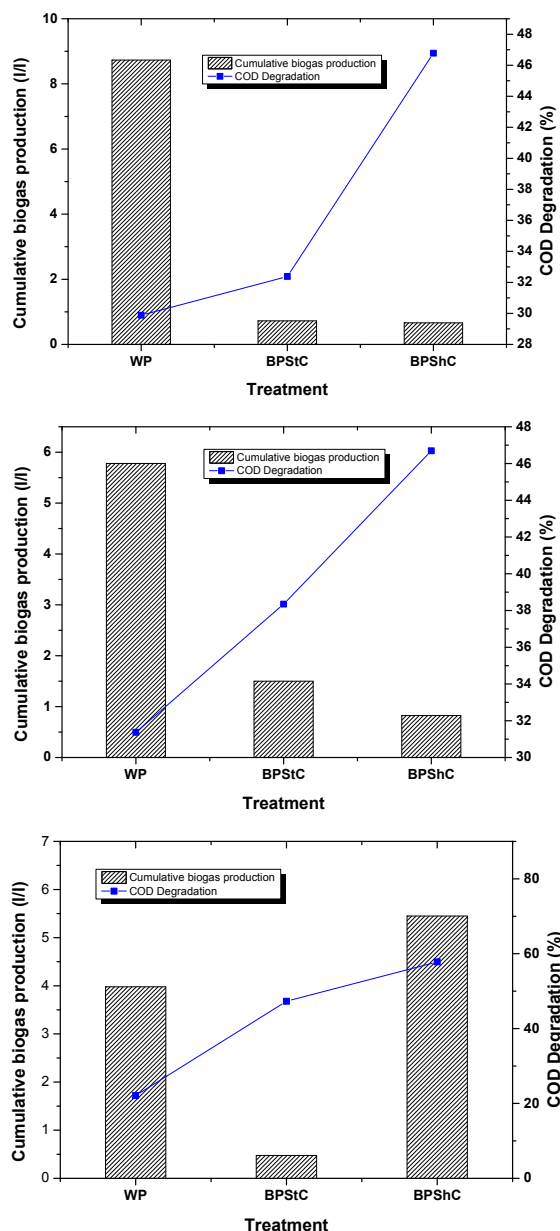


Fig. 8: Effect of different biological pretreatment conditions on a cumulative biogas production and COD degradation efficiency using cellulose concentration of 100 g/l under different C/N ratios (a) 20, (b) 40 and (c) 60.

WP, Without pretreatment; *BPSiC*, Biological pretreatment with static condition; *BPShC*, Biological pretreatment with shaking condition

Effect of cellulose concentration on COD removal and mass balance

The effect of three different cellulose concentrations (10, 55 and 100 g/l) on COD degradation and effluent quality was investigated using a controlled batch activity test. The best performance was obtained by using 10 g/l cellulose while, other initial COD concentrations were recorded low effluent quietly and biogas production. In the same line, there is no significant difference between the soluble by-products concentrations under 55 and 100 g/l which means that the maximum degradation by anaerobic mixed microflora was verified. The decomposition of cellulose involves multiple steps involving three biological bioreaction at least: hydrolysis, acetogenesis and methanogenesis. Clostridium group bacteria are widespread among hydrolyzing cellulose anaerobic organisms as well as genera Bacteroides, Ruminococcus and Cellobacterium (Pavlostathis *et al.*, 1990). The hydrolysis

products of cellulose are converted anaerobically into alcohol, organic acids, hydrogen gas and CO₂ (Timmis *et al.*, 2010). The volatile fatty acids (VFA) concentration especially butyrate/acetate ratio is a key parameter for optimizing and improving bioenergy recovery from the cellulosic waste/wastewater treatment. An example of a syntrophic relationship is a methanogenic culture where the natural, stable processing of anaerobic food chains require the hydrogen transfer from one to another microbe species (Angelidaki *et al.*, 2011). In this study, cellulose was used as a source of energy and carbon sources which has limited to use by a wide range of microflora effectivity. Our results confirmed that the digested sludge mixed microflora offers several advantages for efficient cellulite-bioenergy conversion technology as well as other operational factors. The symbiotic cooperation between bacterial species (Syntrophy) which complement one another by the breakdown of a certain substratum and a mere addition of a cosubstrate or any other co- substrate cannot eradicate such mutual reaction (Song & Clarke, 2009; Timmis *et al.*, 2010). The methane gas was produced during redox reactions involving external inorganic electron acceptors and converts the soluble metabolites such as acetate by acetoclastic processes (Song & Clarke, 2009).

Effect of pretreatment on anaerobic micro-organisms performance

A series of standardized activity experiments were carried out to study the influence of two different biological pretreatment conditions on biogas improvement and COD removal efficiency from cellulosic wastewater treatment. The experimental results reported that the biological pretreatment under shaking cultural conditions produced better results in COD degradation efficiency however, the biogas production was negatively affected as well as the soluble by-products concentrations were significantly decreased. These results suggested that the culture condition played an important role in optimizing and improving the hydrolysis and degradation of cellulose. Therefore, biological pretreatments involve mild conditions and are economically beneficial. However, the drawbacks connected to these pretreatment methods, such as low rates of hydrolysis and long pretreatment times, weigh against their advantages (Song & Clarke, 2009). Other previous study reported that the treated cellulosic wastes with white-rot fungi during 28 days of incubation increased the initial rate of hydrolysis during 10 days of digestion with and without a nutrient-rich medium, while the total methane yield was slightly higher for the nutrient-rich medium after 28 days with white-rot fungi compared with those of the untreated straw.

Conclusion

In this study, a series of standardized batch experiments were carried out to optimize the biogas production from cellulosic-rich wastewater treatment. Three different levels of C/N ratios of 20, 40 and 60 were investigated to assess the impact on the treatment performance. The experimental results showed that the best result was produced under C/N ration of 20 with 80.94 % COD degradation and cumulative biogas production reached 44.55 ml/g COD added. Four different pH levels were studied during cellulosic wastewater treatment and bioenergy conversion. The experimental results showed that the maximum activity and biogas yield were recorded at pH of 8 and an inverse relationship between cellulose concentration and degradation efficiency and/or energy recovery efficiency was observed. As for biological pretreatment, the experimental results showed that the pretreatment under shaking cultural conditions produced better results in COD degradation efficiency however, the biogas production was negatively affected as well as the soluble by-products concentrations were significantly decreased. The experimental results showed that the changing in the soluble by-product concentrations could explain the effect of operational factors and the best conditions were found.

Acknowledgement

The National Research Center, Cairo, Egypt is acknowledged for financial support through project code TT110701

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