The Potential of Acetonitrile Degradation Using Anaerobic Microbial Consortium

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Abstract

Research activities in laboratories produce various types of waste including chemical waste. Acetonitrile is a widely used in laboratory analysis solvent. Acetonitrile can turn into hydrogen cyanide and acetaldehyde which are harmful substances. Therefore, acetonitrile cannot be discharged directly into the environment due to its toxic characteristics. The aim of this research was to reveal the effectiveness of anaerobic degradation of acetonitrile waste in a batch system. The experiments were carried out using mixed culture of microorganisms, and bamboo carrier at the variations in pH and acetonitrile concentration. The reactor was incubated for 28 days at room temperature. Samples were taken periodically for analysis of pH, acetonitrile concentration, TOC, TS, OTS, COD, and biogas production during the study.

The results showed that the pH of the samples was in the range of 4….8 for 28 days. The levels of TS, OTS, TOC, COD and acetonitrile concentration from day-to-day decreased indicating that the degradation of waste by mixed microbial culture took place. The highest biogas production was obtained in the reactor with 5% acetonitrile concentration at pH 7. The greatest degradation efficiency (based on the reduction in COD levels) was 85% obtained at acetonitrile concentration of 5%, pH 4, and using bamboo as a carrier immobilasing material.

Key words: anaerobic degradation, acetonitrile, mixed microbial culture, batch experiments.

1. Introduction

Research activities in the laboratories produce various types of waste, especially chemical waste. Chemical waste is derived from the residual or expired chemicals, the remaining sample materials or remaining materials used in analysis activities both from the preparation stage and the instrument stage, the residues spill, from the remaining packaging to the remaining products of the research process that do not meet regulatory requirements to be directly discharged into the environment. Chemical waste is categorized as hazardous and toxic waste because they are corrosive, carcinogenic, toxic and combustible.

Acetonitrile is a widely used in laboratory analysis solvent. In the Technical Guidelines for Laboratory Waste Management issued by the National Accreditation Committee in 2006 [1], laboratory waste in the form of solvents are hazardous and toxic organic wastes that are flammable. Acetonitrile cannot be discharged directly into the environment due to its toxic properties.

Acetonitrile can turn into hydrogen cyanide and acetaldehyde which are harmful substances. Several studies have been carried out to solve the problem of acetonitrile waste. Acetonitrile waste treatment techniques using ozone and photocatalytic oxidation have been tested by Adjei and Ohta [3], [4], but these techniques require high operational costs. Another research was aimed to develop a bioremidiation technique to degrade acetonitrile, the development of degrading microorganisms began to be used as conducted by Alfani et al. [5] using Brevibacterium imperalis CBS489-74 [6] and Candida guilliermondii CCT 720 [7] using Comamonas testosterone and Acidovorax sp. [8] using a combination of B.subtilis biofilm forming and R. rhodochrous acetonitrile degrading microorganisms.

Based on the results of previous studies it was shown that some species of microbial isolates have a potential to degrade acetonitrile, but the use of mixed cultures or a consortium of microorganisms also had the potential to degrade acetonitrile, nevertheless their use was still limited. T. Li, Liu, Bai, Ohandja, & Wong [9] in their research attempted to degrade acetonitrile using a microbial consortium in anaerobic conditions, the results of the study showed that acetonitrile could be degraded by 69.9% within 4 hours using mixed culture adapted in anaerobic conditions.

In this research, the residual acetonitrile degradation process was carried out in an anaerobic batch system using the inoculum of mixed microbial culture containing a consortium of biogas-producing microorganisms. The purpose of this study was to determine the tolerancy and resistance of anaerobic microorganisms to the concentration of acetonitrile, as well as their degrading ability towards to the acetonitrile substrate. Variations in waste concentration and the pH of the were tested. Bamboo medium is known as breeding ground for microorganisms and is proven to be an effective place for immobilization and growth of microbial biofilm. [9].

2. Materials and Methods

2.1 Inoculum Preparation

The inoculum was taken from two places: the farmhouse of Kab. Bandung and Tofu Waste Treatment Plant in Sumedang, then mixed in propprtion 50-50%The inoculum was incubated in a drum cultivator with the addition of water (1: 1) and molasses as a source of nutrition, then left for 7….14 days while being shaken until gas bubbles appeared and pH became 7. The inoculum was taken as much as 15 L and put into the HDPE plastic jerrycan. The inoculum was added with a 10% glucose monohydrate solution (150 grams of glucose dissolved in 1500 mL water) and 10% molasses of 500 mL. The mixture was exhaled by nitrogen gas (N_2) for 3 minutes at a constant speed.

2.2 Acetonitrile Waste Preparation

The acetonitrile sample was isolated from the pesticide content analysis residue at High Performance Liquid Chromatography (HPLC), the acetonitrile waste was obtained from the process of dissolving the tea leaves. From this process, acetonitrile waste obtained was a mixture with other solvents such as hexane, consisting the following compounds: acetonitrile (70%), n-hexane (20%), methanol (10%), and tea leaf extract (tea and chocolate compounds).The acetonitrile was separated from other solvents by using a pipette based in the differerence in density and then homogenised, so that the acetonitrile stock could be used for the experiments.

2.3 Bioreactor Preparation

The treatment variations in this study were: the reactor at the original pH of the waste sample with acetonitrile concentration variation 5%, 10% and 15%; the pH was adjusted to pH 7 by adding NaOH with variations 5%, 10% and 15%; reactor with bamboo pieces as a immobilasing carrier of mixed microbial culture with concentration variatons of 5% and 15%.

Acetonitrile samples were taken and observed periodically every 7 days from the experimental reactor for analysis: pH measurement, acetonitrile concentration using gas chromatography, TOC (total organic carbon), TS-OTS (total solid - organic total solid), COD (chemical oxygen demand), and biogas production during the experiment.

The batch reactor arrangement is shown in Figure 1. The reactor (first flask) filled with acetonitrile waste and has a working volume of 2 L with a ratio of inoculum:acetonitrile waste solution 1: 1. The second flask is filled with 2.5L of water, while the third one in the image is left empty. The third flask was used to accommodate the displacement of water from the second flask caused by the pressure of the biogas formed in the process of the degradation of acetonitrile in the reactor.

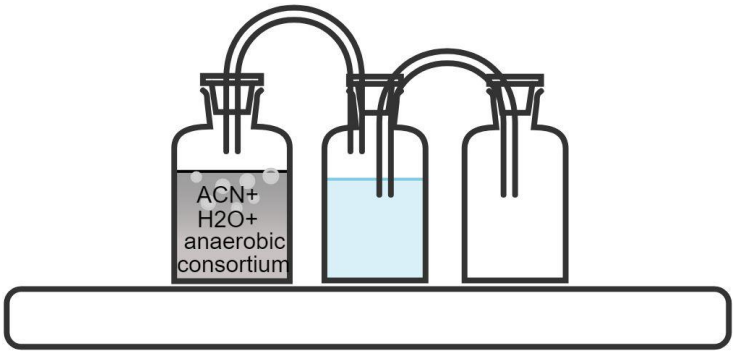

Figure. 1 Batch Reactor in the Experiment

The reactor in Figure 2 has a size of 2.5 L filled with bamboo pieces of the random sizes arranged five layers vertically, each layer containing four bamboo pieces tied with string. The reactor had a working volume of 2 L. After being filled with bamboo, the acetonitrile waste aqueous solution was added to the reactor without pH adjustment with various concentrations (% v/v) 5, 10 and 15% , as well as 0%, then an inoculum was added as much as 1000 mL.

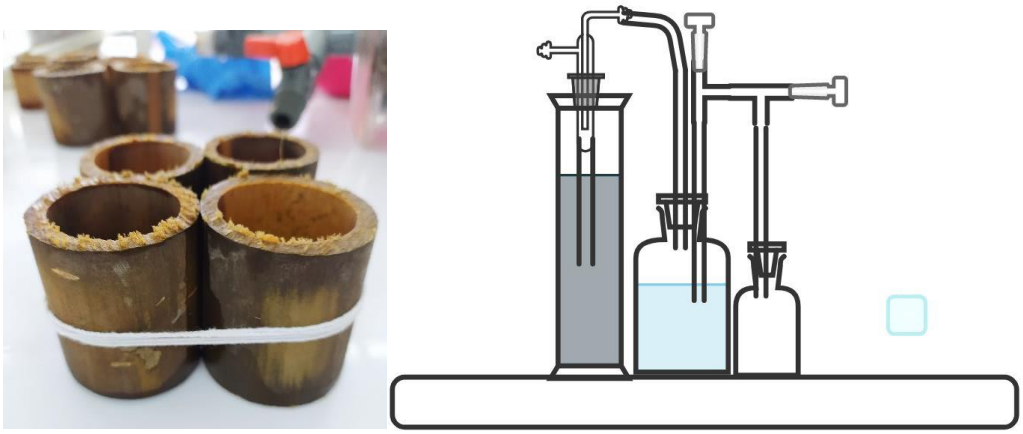

Figure 2. Bamboo Pieces and Reactors with Bamboo as Microbial Immobilising Carrier in Experiment

2.4 Characteristics of Acetonitrile and Acetonitrile Degradation Analysis

The acetonitrile taken from residues of High Performance Liquid Chromatography (HPLC) analysis was characterized in advance by means of the pH, Chemical Oxygen Demand (COD), Total Solids and Organic Total Solids (TS-OTS), Total Organic Carbon (TOC), and acetonitrile concentrations. The reactor was incubated at room temperature (25°C) for 28 days. A sample of 25 mL was taken periodically once a week to measure pH, TS, OTS, TOC, COD, acetonitrile concentrations, and gas production with the same method as with the initial characteristics of acetonitrile.

Total solids (TS) is the total amount of solids presented in water, both dissolved, suspended, and solids that settle in water in the form of organic and inorganic compounds. Total organic solids or total organic solids (OTS) are the total amounts of dissolved or suspended solids which are only organic compounds.

The measurement of TS-OTS sample was carried out at 105 ° C for 24 hours. Then the cup with sample was put into the desiccator for 30 minutes then weighed as weight C. The TS value can be calculated using the following formula: .

$$TS = (C\text{-}A)/(B\text{-}A) \times 100\% \quad (1)$$

A: mass of empty cup

B: wet sample mass

C: dry sample mass.

After weighing, the cup containing the sample is put into the furnace and heated at 550°C for 4 hours (calculated when the furnace temperature reaches 550 ° C) then weighed as a weight D. The OTS value can be calculated using the formula as shown in Equation 2:

$$OTS = (D - A)/(C - A) \times 100\% \quad (2)$$

TOC levels in samples can be measured using a TOC analyzer. The acetonitrile waste sample was diluted 200 times each sample. The TOC (ppm) in the sample was calculated by multiplying the results of the TOC (ppm) in the readings with the dilution factor.

The COD value in the sample was measured using the UV-Vis spectrophotometric method at wavelength of 615 nm. The initial concentration analysis of the acetonitrile sample was carried out using a gas chromatography method with an HP-INNOWax capillary column. The results of the chromatogram have the area on the y-axis and retention time on the x-axis, then the result put into equation y = ax + b. The acetonitrile concentration is calculated by substituting the area value into the Y. The value of x is obtained as the acetonitrile concentration sought and then multiplied by the dilution factor.

3. Results and Discussion

3.1 Inoculum Activation and Adaptation

The aim of inoculum activation is create the optimal conditions for the microorganisms. In the meantime, the inoculum adaptation is very essential step to enchance the efficiency of degradation of organic matters and biogas production. The adaptation of inoculum was carried out in accordance with Rivera et.al. with 20 days adaptation in bioreactor digesting sludge [10], Acosta et al. adaptated 5 liter digesting sludge in 50 days adaptation period and achieved stable values of biogas production, methane content and removal of chemical oxygen demand (COD) [11].

The results of inoculum adaptation showed by biogas composition on the fourth day of incubation are presented in Table 1. The composition indicate that the mixed microbial culture present in the inoculum is in a healthy and active condition and able to assimilate glucose and molasses as a source of carbon and other nutrients.

Table 1. Gas Composition in the Fourth Day of Inoculum Adaptation

Parameter	Value
Methane (CH_4)	35.6%
Carbon Dioxide (CO_2)	28.7%
Oxygen (O_2)	2,1%
Nitrogen (N_2)	33.6%
pH	7.04

The low concentration of methane means that in the microbial activation process the microbial growth was affected by incubation time, substrate concentration and culture for medium acidification [12].

3.2 Acetonitrile Waste Characterization

The acetonitrile waste which was used in this study originates from residual waste of tea leaf extract. The acetonitrile waste contains several chemical compounds such as acetonitrile (70%), n-hexane (20%), methanol (10%), and the remaining content of tea leaf extract. The n-hexane content in the sample has been separated from the other components by liquid extraction. The separation of n-hexane aimed to prevent the formation of other compounds that can inhibit the degradation process of acetonitrile.

The results of measuring the TOC (ppm) of the initial sample is shown in Table 2 . According to Kubsad et al. [13], wastewater containing 100 ppm acetonitrile has COD level between 4.000-6.000 ppm. The initial concentration of the acetonitrile sample which was used in this experiment had a value of 192.088 ppm. COD levels in the sample are compared with the theoretical COD based on the research conducted by Kubsad and colleagues, and known that the COD value in this study is still within the theoretical COD range.

Table 2. Initial Acetonitrile Waste Characterization

Initial Acetonitrile Characterization	
Total Organic Carbon (TOC) (ppm)	Concentration (ppm)
182.564,60	192.088

3.3 Acetonitrile Degradation

Acetonitrile is one of solvent which frequently used in column liquid chromatography, but because of toxic characteristic, the safe disposal of acetonitrile is essential. One of the options is using biological degradation. Microbial degradation of a toxic substance is a challenge because it can be an inhibitor to the microbes, the decomposition can be effective and possible after adaptation of the microbes.

Acetonitrile can be degraded microbially in several steps [14]:

$$\mathrm{RCN} \xrightarrow{\mathrm{H_2O + Nitrile\ Hydratase}} RCONH_2$$

$$\xrightarrow{\mathrm{H_2O + Amidase}} RCOOH + NH_3$$

And then fatty acids can be converted into methane and CO_2.

Figure 3 and 4 illustrate the data obtained over 28 days retention time.

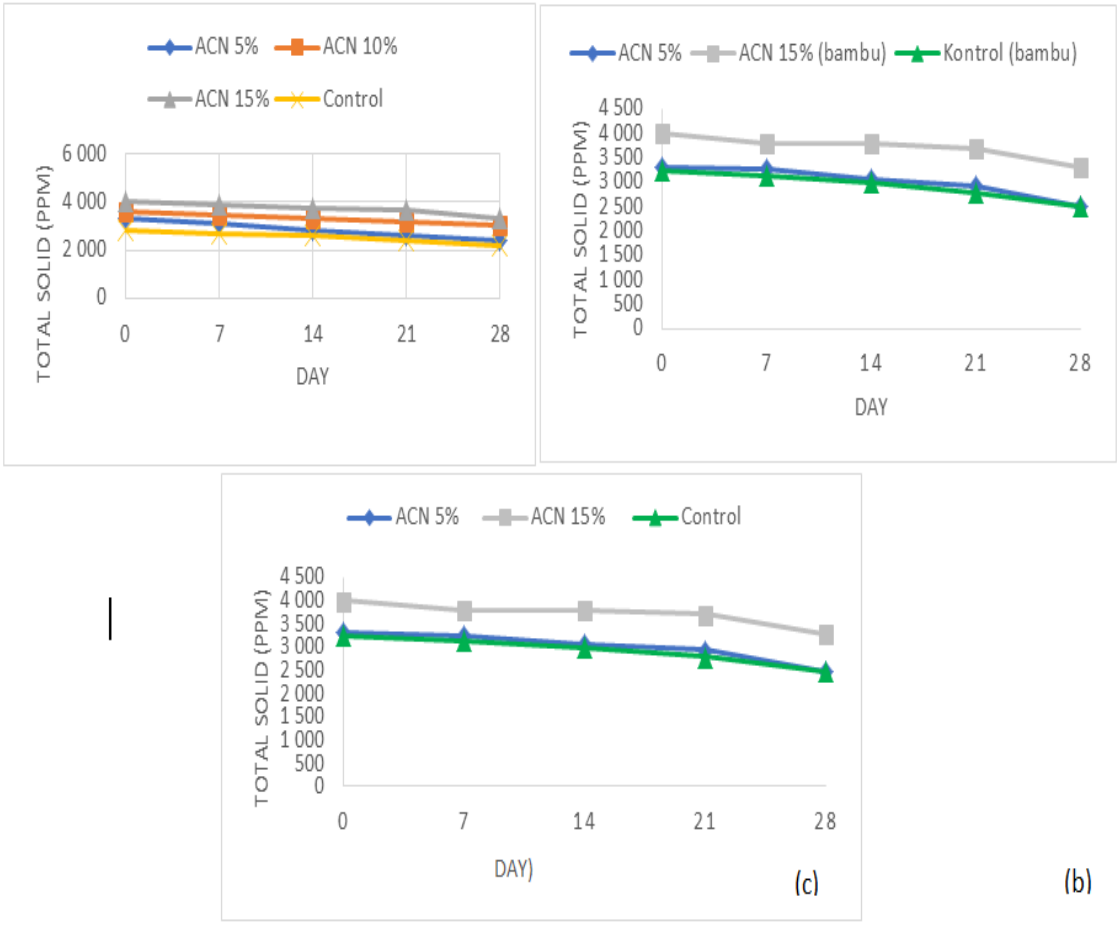

Figure 3. Concentration Total Solid (TS) in 28 days (a) Acetonitrile Reactor without pH Adjusted; (b) Acetonitrile Reactor with pH Adjusted ; (c) Acetonitrile Reactor with Bamboo

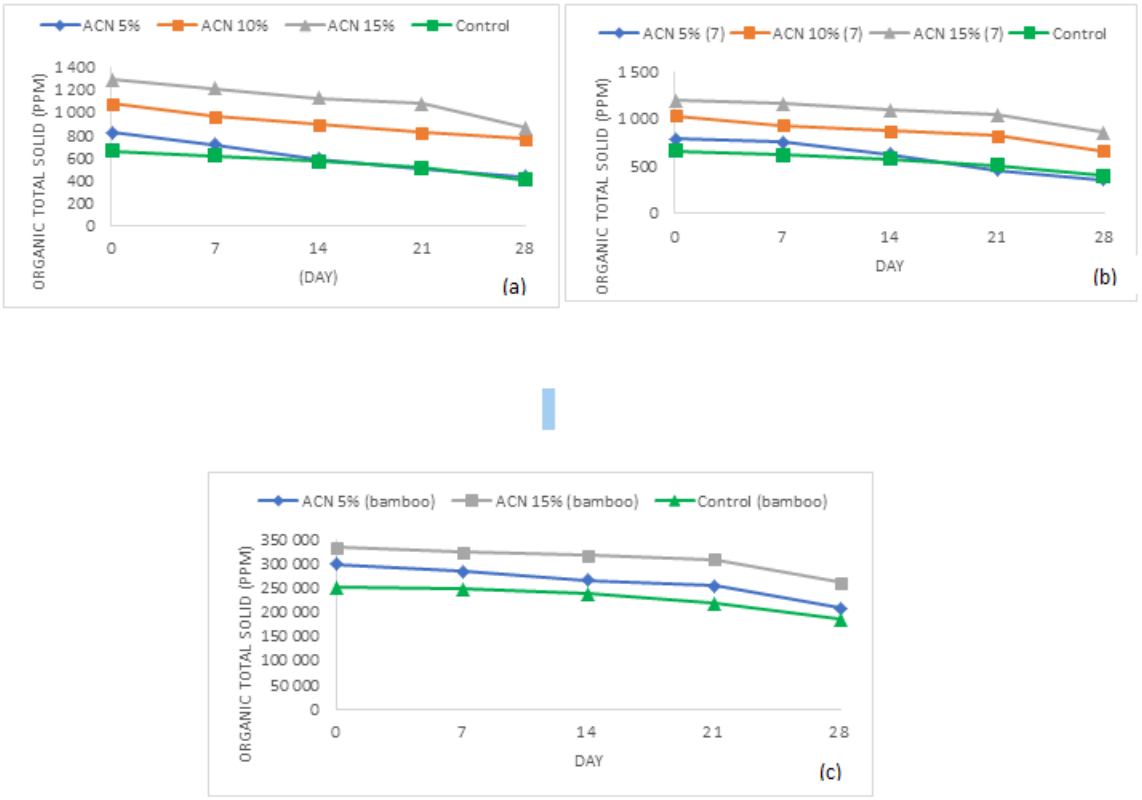

Figure 4. Concentration Organic Total Solid (OTS) in 28 days (a) Acetonitrile Reactor without pH Adjusted; (b) Acetonitrile Reactor with pH Adjusted ; (c) Acetonitrile Reactor with Bamboo

Based on the graphs in Fig. 3 and 4, it was found that the TS and OTS levels of all samples decreased from day 0 to day 28. This is because acetonitrile have been degraded and other compounds such as acetamide, acetic acid, and ammonia are produced. The decrease in TS and OTS levels was not too significant because the anaerobic degradation process took a longer time. In addition, TS levels not only indicate the amount of dissolved or suspended acetonitrile solids, because in the reactor there are also other inorganic compounds, solids or sediments containing microorganisms, sludge, metal oxides etc.

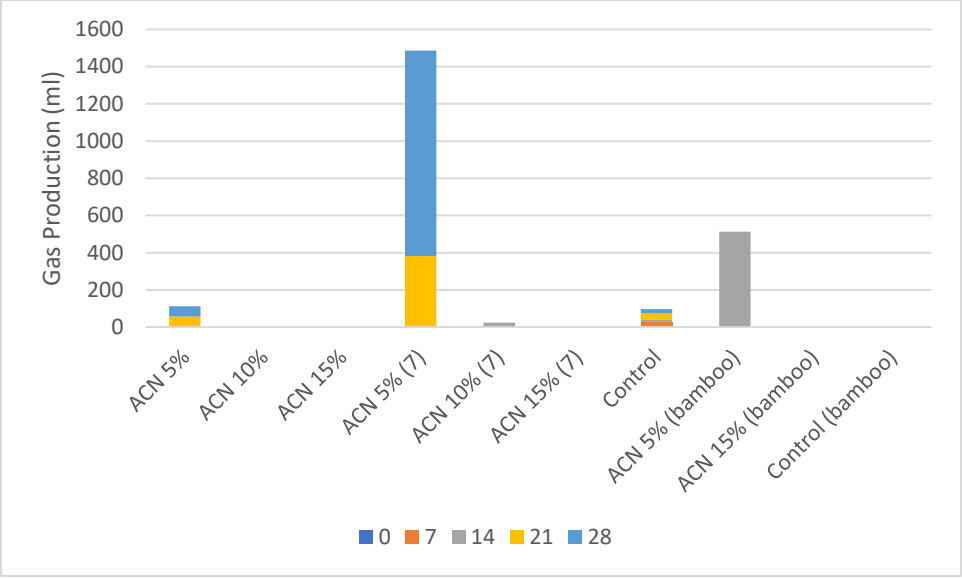

Figure 4. Biogas Production during the Experiment

The microbial selection process starts with a complex mixture of organisms, the different concentrations showed the microorganisms ability to degrade acetonitrile and to get adopted microorganisms. Stress factors in anaerobic digestion can influence the degradation and gas production process [15]. Temperature is critical for the development of anaerobic digestion, in our study we used mesophilic microorganisms with operating temperature between 25-40^{o} C. Gilomen et al in the study of detoxification of acetonitrile stated that hydrolysis stage was possible at room temperature, but it took a time approximately 15 days to reach ACN level 0.02% from 10% of ACN [16].

Another basic essential factor is pH, the optimal hydrolysis stage on anaerobic digesting is between 5 and 6 and for methane production is between 6.5 and 8. Methane production with pH below 6 is inhibited [17]. The effect of pH for anaerobic digestion has been analyzed in this experiment. The results obtained showed that the reactor with 5% acetonitrile and adjusted pH produces higher gas than the other reactors.

Based on the TOC analysis the reactor adjusted with NaOH until pH 7 with 5% concentration of acetonitrile has 18% efficiency of degradation, this is higher than the reactor with normal pH of

acetonitrile (pH 4) where it was 14% with the same concentration of ACN waste. The highest degradation efficiency was achieved in the reactor with bamboo shoots as a immobilizing media and 5% concentration of acetonitrile. The use of bamboo is for support of the biofilm in anaerobic digestion. Immobilized biomass can be advantageous for the degradation of organic matter. In anaerobic wastewater treatment the silicate carrier material with hydrophilic interfaces are generally in use [18].

Table 2. Acetonitrile Degradation Efficiency based on TOC

Sample Code	Degradation efficiency (%)
ACN 5%	14.8017
ACN 10%	11.3375
ACN 15%	12.6537
ACN 5% (7)	18.1568
ACN 10% (7)	13.9346
ACN 15% (7)	11.3077
Control	38.1305
ACN 5% (bamboo)	44.8620
ACN 15% (bamboo)	30.0154
Control (bamboo)	34.1408

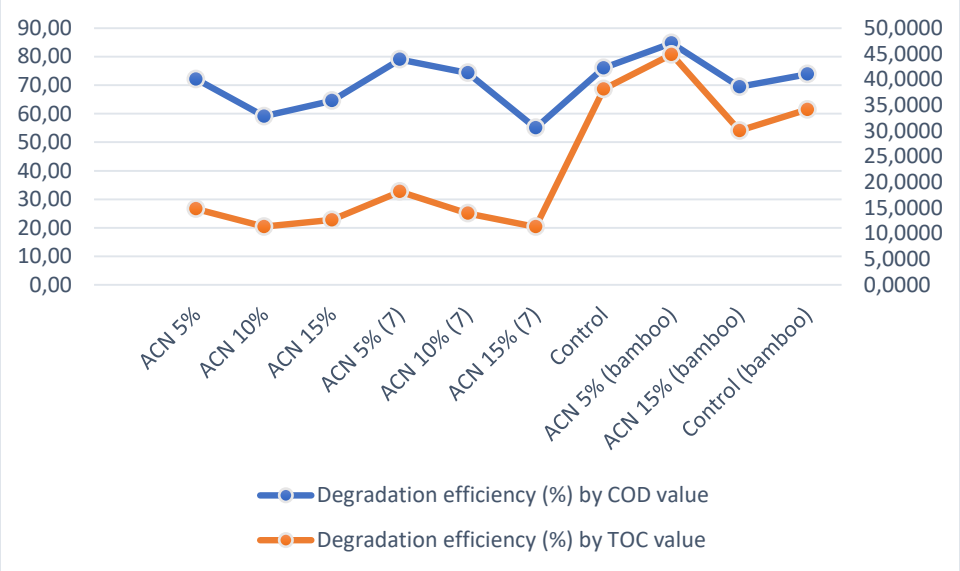

Figure 5. Degradation Efficiency (%) of Acetonitrile by COD and TOC

Table 2 and Figure 5 represent the efficiency of degradation calculated on the basis TOC and COD.

The results show that the highest value of degradation efficiency based on COD was obtained at 85% from the reactor with 5% acetonitrile and pH 4, as well as with the use of bamboo for microbial attachment. The decrease in the value of degradation efficiency based on COD levels has the same tendency as TS, OTS, and TOC levels. The highest value of degradation efficiency based on TOC measurements is 45%, this value was obtained for the reactors with 5% acetonitrile concentration, pH 4, and the use of bamboo as attachment media.

It was not possible to fully degrade the acetonitrile by anaerobic digestion first of all because its inhibiting properties towards the applied microbial consortium. Second reason is the static circumstances of the experiment. Further experiments should be performed using gen-manipulated species, as well as applying a dynamic processing system.

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