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# Anaerobic biodegradability of terephthalic acid and its inhibitory effect on anaerobic digestion

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**Abstract** — The behavior of terephthalic acid (TPA) in anaerobic system has been studied by semicontinuous bioassays under mesophilic condition with artificial TPA production wastewater. The effect of different loading rate of TPA on anaerobic digestion was studied under certain COD loading rate. The results showed that the TPA could be degraded anaerobically within a relatively low range. The degradable concentration of TPA was less than 500 mg/L in the digester, higher concentration of TPA could not be degraded totally and the rate of degradation might decrease with the increase of feed amount. The inhibition is related to both loading rate and accumulated concentration of TPA in the digesters.

**Keywords:** anaerobic biodegradability; terephthalic acid; inhibitory effect; semi-continuous bioassay; accumulated concentration.

## 1 Introduction

Phthalic acid esters (PAEs) have become one of the most main classes of chemicals in industry today. They have been widely used as plasticizes, pesticides, cosmetics, etc. in many major product category: construction, automation, household products, apparel, toys, packaging, and medical products. This results in wide distribution of phthalic acids in water, sediments, soil and plants. And they are relatively persist in degrading. So far, few studies have concentrated on the anaerobic fate of PAEs (Aftring, 1981; Shelton, 1984; Nozawa, 1988). Anaerobiosis usually occurs in the habitat in which oxygen consumption exceeds its supply and is a common phenomenon in natural environments, such as flooded soils, sediments, lagoons, anaerobic fresh and ocean waters, and some groundwaters.

Wastewater from TPA production factories usually contains high concentration of organic compounds. Anaerobic biotechnology perhaps is a suitable method used for treatment of this kind of wastewater. The goals of present study are (1) to investigate the anaerobic biodegradability of TPA; (2) to determine the range of degradable concentration, and (3) to offer theoretical support for the treatment of TPA production wastewater by anaerobic degradation.

## 2 Materials and methods

### 2.1 Apparatus

A schematic diagram of the anaerobic digesters used in this study is shown in Fig. 1. Anaerobic sludge was obtained by acclimating seed sludge to an artificial wastewater containing glucose for 2–6 months, after taking it from the bottom of a settling tank at a wastewater treatment plant in Beijing. Cultivation of the seed sludge for acclimation was carried out anaerobically in a 5L breeder thermostated at 35 ℃ . The bacteria were kept in an active state of growth by removing 80 ml of the culture and restoring the original volume by the addition of raw artificial wastewater everyday.

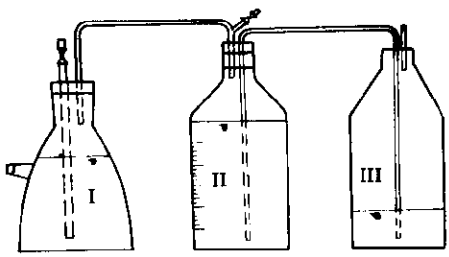


Fig. 1 Schematic diagram of the anaerobic bioassay tests

I. Anaerobic digester II. Gas collector III. Water bottle

### 2.2 Procedure

The temperature of the digesters immersed in a water bath was maintained at 35℃ . There are four digesters in all. Digester 1 to 3 are test digesters feeding with artificial TPA wastewater. Digester 4 is a control unit. The digesters were each charged with 200 g of actively digesting sludge from the breeder and required volume of salt solution, then immediately put into operation. Each day 30 ml digested solution was

**Table 1 Important operating parameters**

| Digester No. | Parameter | Magnitude |
|---|---|---|
| 1 | Test temperature, ℃ | 35± 1 |
| 2 | Test period, d | 27 |
| 3 | Volume of digesters, ml | 500 |
| 4 | Volume of liquid phase, ml | 300 |
| 5 | Volume of seed sludge, g | 200 |
| 6 | Volume of substrate, ml/d | 30 |
| | Seed characteristics | |
| 1 | Total solids, % | 9.41 |
| 2 | Volatile solids, % | 3.97 |

removed and an equal amount of feeding was added into the digesters. The reactors were operated for at least 10 days to obtain reproducible and stable operation before introducing TPA into the feed. The important operating parameters are shown in Table 1.

The artificial wastewater consisted of different concentration of TPA and glucose solution. The ratio of COD:N:P is 200:5:1. The feeding to each digester comprised certain concentration of TPA plus the required volume of glucose nutritious solution, diluted to 30 ml with tap water.

The operating routine consisted in measuring the overnight gas production and shaking the bottles for a few seconds.

2.3 Analyses

The gas was analyzed by SP-2305 gas chromatograph equipped with a glass column packing Propak N and a TCD detector. Pure hydrogen (99.999%) was used as a carried gas at a flow rate of 60 ml/min.

Volatile fatty acids were determined by gas chromatograph with a glass column packing Chromosorb 101 (60－80 mesh) and coating 2% $H_3PO_4$ using a FID detector. Pure nitrogen was used as a carried gas at a flow rate of 78 ml/min.

TPA was tested by Shimadzu UV-3000 photometer at wavelength 240 nm.

Chemical oxygen demand (COD) was tested by HH-1 COD meter.

pH and alkalinity were measured with a pH meter.

All these parameters were analyzed every two days.

## 3 Results and discussion

The operating parameters and the results of the semi-continuous bioassays are shown in Table 2 and Fig. 2, respectively.

As for digester 1 to 3, daily gas production rates decreased with increasing TPA concentration and dropped below 30% for very high TPA concentration. The inhibited system may be recovered gradually after the termination of adding TPA. Methane production rate during digestion also decreased to as low as 30% with increasing TPA concentration. The concentration of total volatile fatty acids was directly proportional to the TPA concentration and even accumulated to 6000 mg/L in the inhibited system.

In this study, inhibition degree were defined according to the decreased percentage of gas production compared with that of the control unit. Four degrees were defined as follows: (1) No inhibitory effect, corresponding to a 5% decrease in gas production. (2) Slightly inhibited corresponding to 5%－20% decrease in gas production.

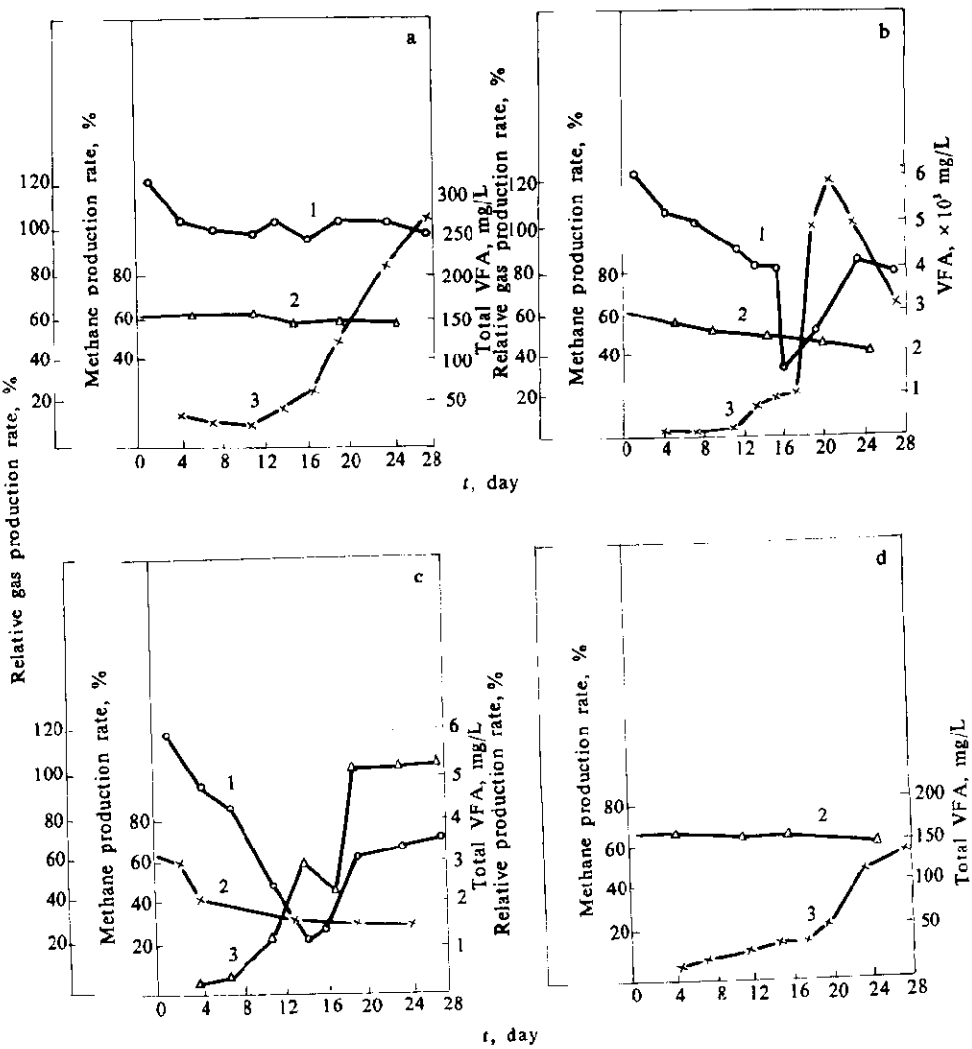


Fig. 2 The result of TPA anaerobic bioassay test

1. relative gas production rate 2. methane production rate 3. total VFA

a, b, c, d are the digesters 1, 2, 3 and 4, respectively.

**Table 2 Operating parameters and the results of the semi-continuous bioassays**

| Test periods | 1<br>1 – 27d | 2<br>1 – 15d | 2<br>16 – 27d | 3<br>1 – 13d | 3<br>14 – 27d | 4<br>1 – 27d |
|---|---|---|---|---|---|---|
| COD in feed, mg/L | 23200 | 26150 | 20150 | 35200 | 20150 | 20150 |
| TPA in feed, mg/L | 2400 | 4800 | – | 12000 | – | – |
| COD/TPA | 10:1 | 6:1 | – | 3:1 | – | – |
| COD volumetric loading rate, g/L • d | 2.32 | 2.61 | 2.02 | 3.52 | 2.02 | 2.02 |
| COD sludge loading rate, g/gVS • d | 0.088 | 0.099 | 0.076 | 0.133 | 0.076 | 0.076 |
| TPA volumetric loading rate, g/L • d | 0.24 | 0.48 | – | 1.20 | – | – |
| TPA sludge loading rate, g/gVS • d | 0.009 | 0.018 | – | 0.048 | – | – |
| COD removal rate, % | 81.7 – 94.0 | 83.2 – 91.3 | 45.5 – 80.0 | 60.2 – 84.3 | 31.3 – 58.0 | 97.8 – 98.8 |
| Tested TPA, mg/L | 240 – 1945 | 1489 – 3602 | 1965 – 3602 | 3335 – 7750 | 3450 – 6840 | – |
| TPA removal rate, % | 27.3 – 90.0 | 25.8 – 68.4 | – | 43.0 – 72.2 | – | – |
| Gas production rate, % | 98.0 – 120.0 | 80.0 – 120 | 30.0 – 80.6 | 40.0 – 116.0 | 20.0 – 69.1 | 100.0 |
| Methane production rate, % | 58.3 – 61.4 | 48.0 – 59.0 | 40.0 – 45.0 | 39.8 – 57.0 | 29.8 – 30.8 | 55.0 – 65.0 |
| Substrate methane production rate, ml/gCODr • d | 279 – 323 | 152 – 318 | 115 – 227 | 123 – 278 | 73.7 – 168.0 | 308.0 – 336.0 |
| Relative methane production rate, % | 94 – 122 | 78 – 123 | 27.5 – 70.2 | 33 – 111 | 14.8 – 50.2 | 100.0 |
| VFA, Total VFA | 20 – 267.9 | 18.5 – 916.2 | 1090 – 5000 | 121.3 – 2856 | 2120 – 5267 | 7.3 – 134.0 |
| Acetic acid, mg/L | 2.5 – 28.0 | 0.0 – 60.0 | 230.0 – 527.0 | 0.0 – 328.5 | 360 – 738.5 | 6.0 – 27.0 |
| Propionic acid | 7.5 – 223.9 | 18.5 – 846.2 | 580.0 – 194.0 | 121.3 – 260 | 80 – 27.5 | 1.3 – 100.0 |
| Butylic acid | 0.9 – 6.0 | 0.0 – 5.0 | 210 – 2629 | 0 – 1080 | 1020 – 3808 | 0.0 – 5.0 |
| Valeric acid | 0.0 – 10.0 | 0.0 – 5.0 | 70.0 – 1650.0 | 0.0 – 1133.5 | 660.0 – 693.3 | 0.0 – 2.0 |
| pH value | 6.9 – 7.6 | 6.4 – 7.3 | 4.8 – 6.4 | 5.0 – 7.3 | 4.3 – 5.2 | 7.0 – 7.6 |
| Dry sludge weight, gVS | | 7.94 | | | | |

(3) Significantly inhibited, corresponding to 20% – 40% decrease in gas production.
(4) Seriously inhibited, corresponding to greater than 40% decrease in gas production.

3.1 The anaerobic biodegradability of TPA

From Table 2, we can see that the TPA concentrations in digesters were increased gradually, e. g., one in digester 3 to as high as 7750 mg/L. It means that a large amount of TPA was not degraded anaerobically but accumulated gradually. Such accumulated concentration can be calculated as follows:

$$C_n = A\,[1 - (1 - Ve/Vo)^n],$$

where, $C_n$ is the accumulated concentration of TPA in the $n$th day (mg/L); $A$ is the TPA concentration in the feeding (mg/L); $Ve$ is the volume of the feeding (ml); $Vo$ is the volume of the liquid phase in the digesters (ml).

**Table 3 Anaerobic degradation of TPA**

| | No | t, d | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 4 | 7 | 11 | 13 | 15 | 19 | 23 | 27 |
| TPA in feed, mg/L | 1 | 240 | 240 | 240 | 240 | 240 | 240 | 240 | 240 | S |
| | 2 | 480 | 480 | 480 | 480 | 480 | S | S | S | S |
| | 3 | 1200 | 1200 | 1200 | 1200 | 1200 | S | S | S | S |
| Tested TPA conc., mg/L | 1 | | 637.5 | 1454 | 1430 | 1596 | 1701 | 1855 | 1905 | 1945 |
| | 2 | | 1489 | 2385 | 3200 | 3330 | 3602 | 2370 | 2015 | 965 |
| | 3 | | 3335 | 5340 | 6130 | 7750 | 6840 | 4350 | 3850 | 3450 |
| Calculated TPA conc., mg/L | 1 | | 825.4 | 1252.1 | 1646.9 | 1789.9 | 1905.9 | 2075.8 | 2187.3 | 2260.4 |
| | 2 | | 1650.7 | 2504.2 | 3293.7 | 3579.9 | 3871.7 | | | |
| | 3 | | 4126.8 | 6260.4 | 8234.3 | 8949.8 | 9520.3 | | | |
| TPA reduced amounts, mg/L | 1 | | 187.9 | 202.0 | 216.0 | 194.0 | 205.0 | 220.8 | 282.0 | 315.0 |
| | 2 | | 161.7 | 119.2 | 273.7 | 249.9 | 269.7 | | | |
| | 3 | | 791.8 | 920.4 | 2104.3 | 1199.8 | 2689.3 | | | |
| $CH_4$ calculated amount, ml | 1 | | 26.8 | 28.8 | 30.8 | 27.6 | 29.2 | 31.5 | 40.2 | 44.9 |
| | 2 | | 23.0 | 27.0 | 39.0 | 35.7 | 38.5 | | | |
| | 3 | | 112.9 | 131.2 | 299.5 | 169.0 | 383.0 | | | |
| $CH_4$ tested amount, ml | 1 | | 8.3 | 18.9 | 18.6 | 20.8 | 22.1 | 24.1 | 24.8 | 25.3 |
| | 2 | | 15.1 | 24.2 | 32.5 | 33.8 | 36.5 | | | |
| | 3 | | 29.0 | 46.4 | 53.2 | 67.3 | 59.4 | | | |
| TPA reduction rate, % | 1 | | 73.4 | 39.4 | 40.4 | 31.9 | 27.3 | 22.7 | 20.6 | 19.0 |
| | 2 | | 69.0 | 50.3 | 33.3 | 30.6 | 25.0 | | | |
| | 3 | | 72.2 | 55.5 | 48.9 | 35.4 | 43.0 | | | |

S: Stop operation

TPA reduced amount = Calculated TPA conc. − Tested TPA conc.

The different amount between the calculated value and the actually measured TPA concentration is close to the amount of being degraded TPA in the reactors. The

results are shown in Table 3.

The tested TPA concentrations were always less than the calculated ones. It means that some amount of TPA were degraded by anaerobes to methane and carbon dioxide via volatile fatty acid. But the amount of TPA reduction was not transferred to methane totally as the result of the fact that VFA were accumulated gradually. For example, the methane production in digester 3 was much lower than the theoretically calculated amount, corresponding to 60% decrease in methane gas production rate. At the same time, the total VFA value increased to 5000 mg/L and the pH value dropped to 4.3. This digester system was seriously inhibited.

3.2 The effect of TPA accumulated concentration on anaerobic digestion

The effect of TPA accumulated concentration on methane production rate is shown in Fig. 3.

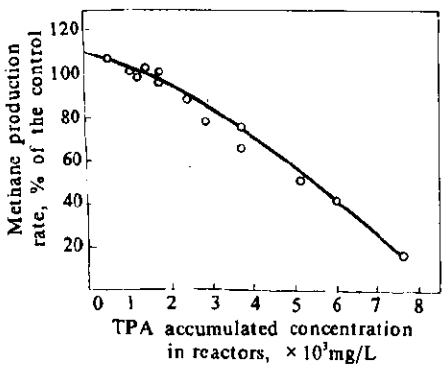


Fig. 3 The effect of TPA accumulated concentration on methane production rate

It appears that one of the most significant consideration in TPA inhibition in anaerobic digester systems is the TPA accumulated concentration in the systems. Under certain COD and TPA loading rates, the same accumulated TPA concentration resulted in the same inhibitory degree in different digesters. Chou and Holliger (Chou, 1978; Holliger, 1988) pointed out that the aromatic compounds in water are toxic to cells. The result of our study also showed the toxicity of TPA to anaerobes, especially to methane production bacteria (MPB). Our conclusions are as follows: (1) The anaerobic systems will not be affected when TPA accumulated concentration is below 1500 mg/L.(2) The anaerobic systems will be slightly inhibited, corresponding to methane production rate in the range of 80%－95%, when the TPA accumulated concentration is below 3000 mg/L.(3) The digester systems will be significantly inhibited when TPA accumulated concentration is in the range of 3000－5000 mg/L.(4) The digester systems will be seriously inhibited, corresponding to 40% decrease in methane production rate, when the TPA accumulated concentration is greater than 5000 mg/L.

3.3 The change of volatile fatty acids in digesters

The volatile acids analysis has been used successfully to determine abnormal

digestion conditions. A rapid increase of total VFA concentration indicates that the MPB are not keeping pace with the volatile acids production and it is the main character of the unbalanced digestion systems. The result of change of VFA is shown in Fig. 4.

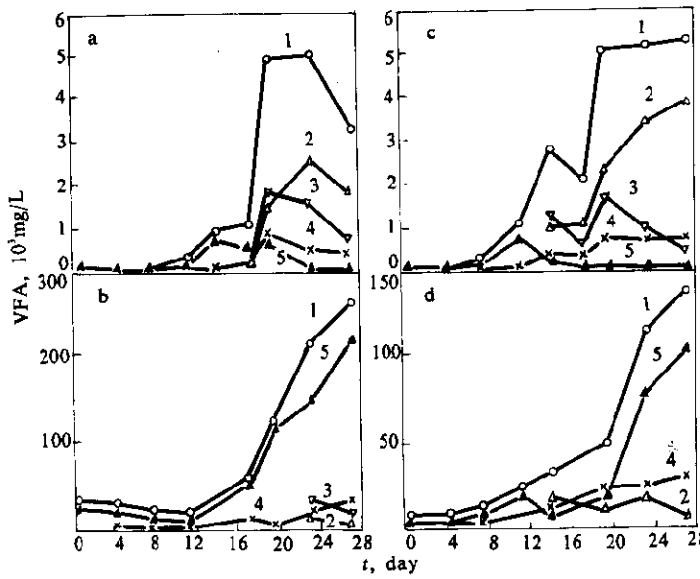


Fig. 4 The change of VFA in TPA degradation test
1. Total VFA 2. Butyric acid 3. Valeric acid
4. Acetic acid 5. Propionic acid

The amount of butyric acid and valeric acid increased when total VFA concentration was greater than 1000 mg/L and pH value dropped to 5. For example, the amount of butyric acids contributed more than 70% and 50% of the total VFA in digester 2 and digester 3 respectively. So the presence of butyric acid may be as a symbol of the unbalanced digester systems.

3.4 The effect of anaerobic sludge loading rate on TPA digestion

The dry weight of anaerobic sludge in another significant consideration in anaerobic digester systems. The sludge loading rate of TPA can be used to evaluate the inhibitory degree. As shown in Table 4, the sludge loading rate of TPA is directly proportional to the inhibitory degree. The sludge loading rate of TPA should be less than 9.06 g/kg vs. in order to maintain the normal running of the anaerobic systems.

**Table 4 The effect of TPA sludge loading rate on inhibitory degree**

| No. | Sludge, gVS. | TPA in feed per day, mg/L | g/kgVS. | TPA accumulated conc., mg/L | g/kgVS. | Decreased in $CH_4$ Production rate, % | Inhibitory degree |
|---|---|---|---|---|---|---|---|
| 1 | 7.94 | 240 | 9.06 | 240 – 1955 | 9.06 – 73.87 | <2 | No effect |
| 2 | 7.94 | 480 | 18.12 | 480 – 3000 | 18.12 – 113.0 | <20 | Significantly |
| 3 | 7.94 | 1200 | 45.31 | 1200 – 5000 | 45.31 – 188.9 | <40 | Seriously |
| | | | | 5000 – 7750 | 188.9 – 292.8 | 40 – 85 | |

## 4 Conclusions

The anaerobic biodegradable concentration of TPA was below 500 mg/L in the condition of this study. Higher concentration of TPA cannot be degraded totally, and would cause some inhibition to anaerobic system.

The accumulated concentration and the sludge loading rate of TPA are the two main characters to evaluate the inhibitory degree.

It is better to treat the TPA wastewater with certain amount of other carbon sources so as to maintain the normal running of the anaerobic systems.

Semi-continuous bioassay tests are simple to operate and can give useful results quickly.

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