

## Nitrite and nitrate as electron acceptors for biological sulphide oxidation

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### Abstract

Autotrophic denitrification and denitrification with sulphide were investigated at bench scale. The results indicated that the process allows to achieve complete sulphide removal in a wide range of operating conditions (SRT and T). Tested sulphide loads were estimated from the H<sub>2</sub>S produced in an anaerobic digester treating vegetable tannery primary sludge; nitrogen loads originated from the nitrification of the supernatant. Nitrogen removal efficiencies higher than 80% were reached in all the tested conditions once steady state was reached. Nitrite and thiosulphate tend to accumulate especially in the presence of variable environmental conditions.

### Keywords

Anoxic processes, denitrification, denitrification, nitrate, nitrite, sulphide biological oxidation.

### INTRODUCTION

Sulphide management represents a major problem in many WWTPs and specific industrial areas as the Tuscan tannery district (Italy), the second largest in Europe, in which more than 5300000 m<sup>3</sup> per year of tannery wastewater are produced. In this context, Cuoiodepur WWTP (San Miniato, Pisa-Italy) treats almost exclusively vegetable tannery wastewaters characterized by high COD concentration (12-23 gO<sub>2</sub> L<sup>-1</sup>), Suspended Solids (6-31 gSS L<sup>-1</sup>), Ammonium (0.12-0.25 gN-NH<sub>4</sub>-L<sup>-1</sup>), Chlorides (0.3 to 8 g L<sup>-1</sup>) and Sulphate (1.7-2.7 gSO<sub>4</sub>- L<sup>-1</sup>) (Mannucci et al., 2010), and produce fertilizers from sludge after thermal drying and mixing with by-product of industrial tannery process.

Since European regulation on land application is becoming increasingly stringent, it is important to evaluate alternative options such as anaerobic digestion of primary sludge (Dhayalan et al., 2007; Zupancic and Jemec, 2010; Kameswari et al., 2012). However, the presence of tannins is critical for methanogenic bacteria and may affect the outcome of the competition between sulphate reducing bacteria (SOB) and methanogens (Mannucci et al., 2014).

The SOB activity causes high sulphide concentration in the produced biogas and make sulphide removal mandatory before methane utilization.

Up today, chemical scrubbing is the most established technology for H<sub>2</sub>S removal in WWTPs (Gabriel et al., 2004) due to several advantages: short contact time (1.3 to 2 s), low capital cost, easy operation and the possibility of treating highly variable loads. However, chemical scrubbing requires large amounts of chemicals, primarily NaOH, that increase both the operating costs and carbon footprint of the treatment. Biological processes application, based on the use of Biotrickling Filters (BTFs) do not require the use of chemicals, can remove H<sub>2</sub>S under varying operating

conditions and require only a few days for biological process start-up (Wu et al., 2001; Namini et al., 2008). However, due to the presence of the methane, conventional aerobic biological process for sulphide oxidation can not be applied for the biogas treatment.

The use of alternative electrons acceptors would make the use of BTF technology more favorable for the biogas produced from anaerobic digestion of tannery industrial sludge. The supernatant originated from the anaerobic digestion is characterized by high ammonia concentration (up to 900 mg N-NH<sub>4</sub><sup>+</sup> L<sup>-1</sup>); its nitrification in a side stream separated from the main treatment train will allow the production of liquid streams with high nitrate and/or nitrite concentration that could be used as electron acceptor in autotrophic denitrification process.

Despite autotrophic denitrification with sulphide as electron donor has been investigated by several researchers with the main applicative purpose of applying it in the treatment of liquid streams (Lu et al., 2009), sulphide removal through denitrification was recently tested on biogas and other gaseous streams (Kleerebezem and Mendez, 2002).

In order to achieve the primary objective of hydrogen sulphide removal, the influent ratio between sulphur and nitrogen has to be considered; nonetheless, it is important to highlight that the stoichiometry of the process is flexible, since sulphide can be oxidized either to elemental sulphur, thiosulphate or sulphate and nitrogen can be used as either nitrite or nitrate, with the result of significantly widening the range of potentially suitable S/N ratio values. Moreover, the successful application of a biotrickling filter for biogas treatment, depend on the possibility of controlling biomass growth (Mannucci et al., 2012). Both of the above mentioned issues definitely depend on the bioprocesses stoichiometry and kinetics, which is, to the best of our knowledge, not clearly quantified in all the reactions of interest. For instance, most of previous work on denitrification with sulphide focused on nitrate as electron acceptor; on the contrary, the use of nitrite as electron acceptor, despite some exception (Morales et al., 2012), still need to be deepened. Moreover, most of previous work on the kinetics and stoichiometry of sulphur compounds oxidation, were carried out with thiosulphate as electron donor (Artiga et al., 2005), while very few tests (Munz et al., 2009) have been carried out with sulphide due to the difficulty of using a strongly volatile and, at the same time inhibiting, substrate. This work is aimed at investigating denitrification and denitritation with sulphide, in the presence of typical S/N ratio that can derive from the anaerobic digestion of primary sludge of tannery wastewater.

The present experimentation have been conducted at CER<sup>2</sup>CO Lab located at the Cuoidepur WWTP. Technical feasibility of the application of anaerobic digestion process to tannery primary sludge and of biological sulphide removal from gaseous streams through a MBBTF (Moving Bed Biotrickling Filter) are the subject of two currently active projects at the plant and financed respectively by the Tuscany Region (Meta POR 2007-2013) and by the European Community (Life Env/IT/075 Biosur).

Two identical bench scale SBR reactors (R1 and R2) were fed with liquid influent on the hypothesis that all the H<sub>2</sub>S produced by the anaerobic digestion will completely absorbed in the treatment sections before denitrification and the ammonia from the digester completely oxidized into nitrate (R1) or nitrite (R2).

## **MATERIALS AND METHODS**

Two identical sealed sequencing batch reactors (SBR), namely R1 and R2, with working volume of 3.2 L and HRT of 24 h, were used (Figure 1).

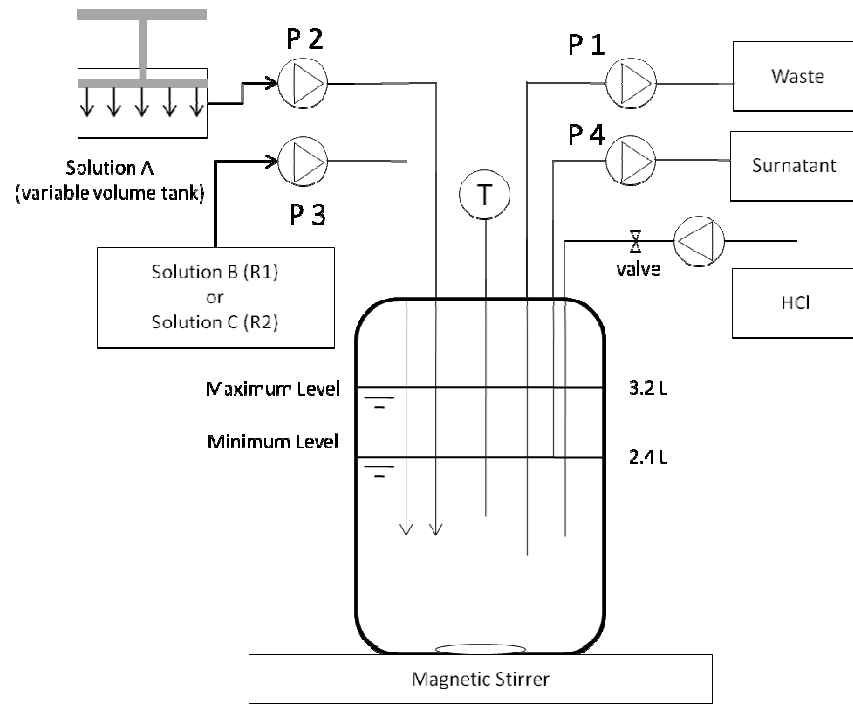


Figure 1 – Schematic of the used bench scale SBR

The reactors were fed with two distinct peristaltic pumps with sulphide (Solution A) with a solution of micronutrients and either nitrite or nitrate (Solution B or C). R1 was operated for 160 days, while R1 biomass was used as inoculum for R2 after 100 days. R1 Experimentation was divided in three phases with different operational conditions as reported in Table 1.

Phase		Start up	I	II	III	III
Parameter	Units	R1				R2
Duration	d	0-25	25-45	46-91	92-160	100-160
$S_{IN}$	g S-HS <sup>-</sup> d <sup>-1</sup>	34-340	340	550	550	214
	g N-NO <sub>3</sub> <sup>-</sup> d <sup>-1</sup>	65-250	250	350	350	-
$N_{IN}$	g N-NO <sub>2</sub> <sup>-</sup> d <sup>-1</sup>	-	-	-	-	205
SRT	d	.*	.*	20	5	13
S/N	g S (g N) <sup>-1</sup>	0.52-1.36	1.36	1.57	1.57	1.05

Table 1 – Operational conditions in R1 and R2

Solution A was stored in the absence of headspace, that is, in a storage tank with a variable volume, and at pH =10 to minimize the desorption of hydrogen sulphide. For Solution A NaHCO<sub>3</sub> (1.24 g L<sup>-1</sup>) and Na<sub>2</sub>S·3H<sub>2</sub>O (0.41 g L<sup>-1</sup>) diluted in demineralised water were used; for Solution B and C Na<sub>2</sub>HPO<sub>4</sub> · 2H<sub>2</sub>O (0.66 g L<sup>-1</sup>); KH<sub>2</sub>PO<sub>4</sub> (0.52 g L<sup>-1</sup>); NH<sub>4</sub>Cl (0.05 g L<sup>-1</sup>); MgSO<sub>4</sub> · 7H<sub>2</sub>O (0.063 g L<sup>-1</sup>); KNO<sub>3</sub> (1.63 g L<sup>-1</sup>; B only) and NaNO<sub>2</sub> (0.96 g L<sup>-1</sup> C only) were diluted in tap water.

The cycle phases for both reactors were as follow: feeding (30 min); mixing (60 min); settling (240 min); decant (30 min). The excess biomass was removed during the last 2 min of the mixing phase.

The reactors were maintained at pH between 7 and 8 through the dosage of an HCl solution and at room temperature (between 18 and 28 °C).

Samples were collected three times a week and the following parameters were monitored: COD (soluble and total), VSS (Volatile Suspended Solids), TSS (Total Suspended Solids), N-NO<sub>2</sub><sup>-</sup>, N-NO<sub>3</sub><sup>-</sup>, S-SO<sub>4</sub><sup>2-</sup>, Sulphide, Total Sulphur, T, pH. Thiosulphate and elemental sulphur, were estimated indirectly. Soluble and total COD, TSS and VSS have been analyzed according to IRSA-CNR (Italian Institute of Water Research-National Research Council) methods. S-SO<sub>4</sub><sup>2-</sup>, Sulphide, Nitrite and Nitrate have been measured through ionic chromatography (ICS1000, Dionex, Sunnyvale - U.S.A.) while total sulphur have been measured using plasma spectrophotometry (ICP-OES, Agilent Technology, Santa Clara – U.S.A.). A portable Hach-Lange (Berlin, Germany) probe was used to measure the pH twice a day in the effluent. Elemental sulphur and thiosulphate have been estimated on the results of total and soluble COD in the wasted mixed liquor. Thiosulphate remains in soluble form and contributes to the soluble COD while elemental sulphur remains in particulate and colloidal form and its contribution to the total COD have been estimated on the basis of the difference between total and soluble COD. Thiosulphate and elemental sulphur concentration in the reactors have been estimated according to Eq. 1 and Eq. 2:

$$S_{thiosulphate} = \left( COD_S - N_{nitrite} \frac{gCOD}{gN_{nitrite}} \frac{gCOD}{gS_{thiosulphate}} \right) \frac{MW_{sulphur}}{MW_{thiosulphate}} \quad \text{Eq. 1}$$

$$S_{elementalsulphur} = \left( COD_P - \frac{VSS}{TSS} \frac{gCOD}{gSSV} TSS \right) \frac{gCOD}{gS_{elemental\ sulphur}} \quad \text{Eq. 2}$$

Where MW = molecular weight.

## RESULTS AND DISCUSSION

In the last two years, an average of 1200 m<sup>3</sup> d<sup>-1</sup> of sludge have been wasted from primary settler of the Cuoiodepur WWTP and sent to a thickener to reduce water content and double the solids concentration. The implementation of the anaerobic digestion phase plan to treat all the thickened primary sludge in an anaerobic digester with an SRT of 15 d. On the hypothesis to treat 600 m<sup>3</sup> d<sup>-1</sup> of thickened primary sludge, a total volume of 9000 m<sup>3</sup> is required.

The S-SO<sub>4</sub><sup>2-</sup> concentration in the primary sludge have been monitored daily for more than 250 days (from January 28<sup>th</sup> to November 17<sup>th</sup> 2013). The average sulphate concentration and the S-SO<sub>4</sub><sup>2-</sup>/S<sub>tot</sub> were 655±120 mg L<sup>-1</sup> S-SO<sub>4</sub><sup>2-</sup> and 0.96, respectively.

On the hypothesis that all the sulphate will be reduced into sulphide due to sulphatereduction within the digester, a daily production of 393±72 g S-H<sub>2</sub>S d<sup>-1</sup> is estimated.

For the evaluation of the ammonia concentration in the supernatant of the anaerobic digester, monitoring data of a pilot scale anaerobic digester fed with 100% of Cuoiodepur WWTP primary sludge have been considered. An average N-NH<sub>4</sub><sup>+</sup> concentration of 700±100 mg L<sup>-1</sup> (data not shown) and an S/N ratio for the autotrophic denitrification of 0.95 gS gN<sup>-1</sup> have been calculated.

An S/N ratio of 1.3 constitutes the worst estimated conditions (maximum sulphide and minimum nitrate/nitrite) to the main objective of complete sulphide removal.

### R1 performance evaluation

During the whole experimentation sulphide removal efficiency (RE) in R1 was always higher than 99% (data not shown). However, a sulphur mass balance based on a complete sulphide oxidation into sulphate does not explain observed RE. The loss of sulphur estimated from the difference between theoretical sulphate production from a complete sulphide oxidation and experimental

sulphate production was 32% and 30% during phase II and phase III, respectively. The analysis of R1effluent in steady state conditions confirms the presence of intermediate sulphur compounds (Table 2).

Reactor	Parameter	IN (mg d <sup>-1</sup> )	S <sub>tot,IN</sub> (mg d <sup>-1</sup> )	OUT (mg d <sup>-1</sup> )	S <sub>tot,OUT</sub> (mg d <sup>-1</sup> )	Diff. (%)
R1 Phase II	S-SO <sub>4</sub> <sup>2-</sup>	109 ± 20	662	552 ± 49	645	2.6
	S-S <sup>2-</sup>	552 ± 32		0		
	S-S <sub>2</sub> O <sub>3</sub> <sup>2-</sup>	0		92.5		
	S-S <sub>0</sub>	0		0		
R1 Phase III	S-SO <sub>4</sub> <sup>2-</sup>	105 ± 18	695	576 ± 80	647	6.9
	S-S <sup>2-</sup>	589 ± 54		0		
	S-S <sub>2</sub> O <sub>3</sub> <sup>2-</sup>	0		64.6		
	S-S <sub>0</sub>	0		6.4		
R2	S-SO <sub>4</sub> <sup>2-</sup>	101 ± 10	434	405 ± 24	477	9.1
	S-S <sup>2-</sup>	332 ± 21		0		
	S-S <sub>2</sub> O <sub>3</sub> <sup>2-</sup>	0		72 ± 8		
	S-S <sub>0</sub>	0		0		

Table 2 – Sulphur mass balance in R1 and R2 in steady state conditions

Nitrate removal efficiency and the ratio between accumulated N-NO<sub>2</sub><sup>-</sup> and reduced N-NO<sub>3</sub><sup>-</sup> (Figure 2) indicates an incomplete denitrification.

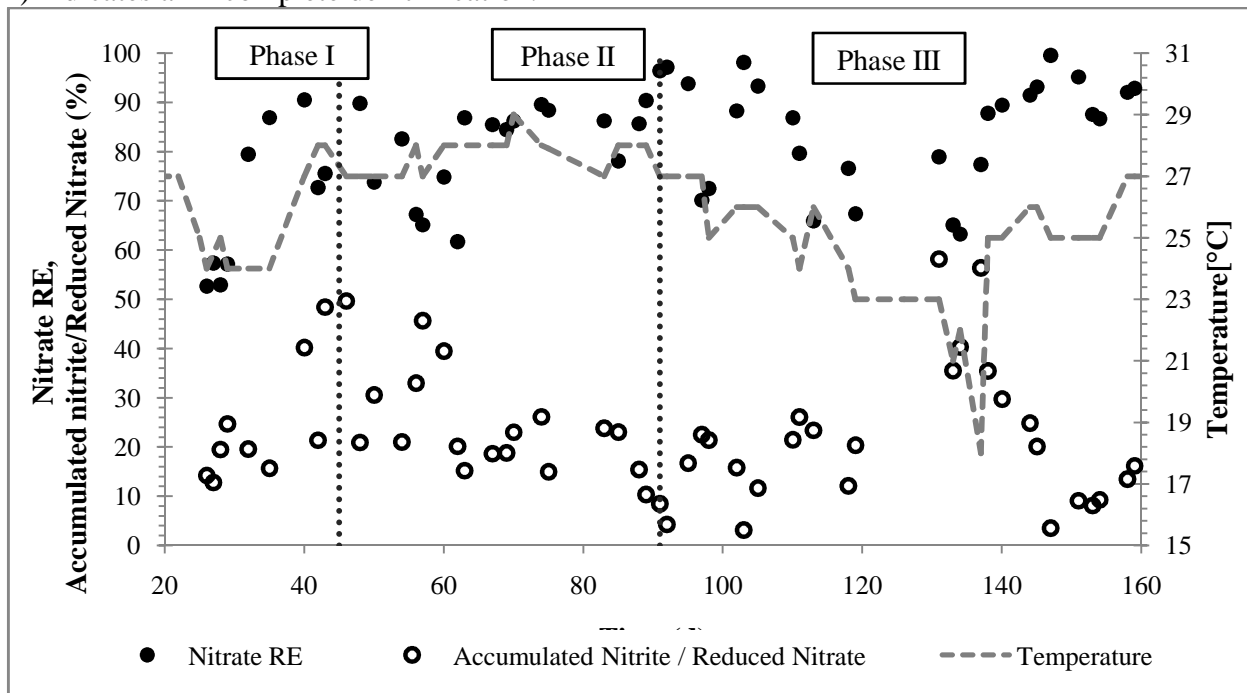
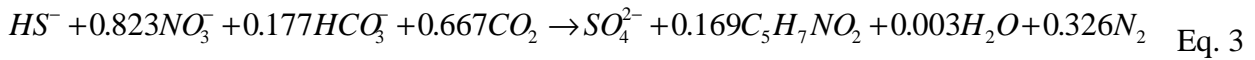


Figure 2 - Nitrate removal efficiency and ratio between accumulated N-NO<sub>2</sub><sup>-</sup> and reduced N-NO<sub>3</sub><sup>-</sup> in R1

During phase II, an initial decrease of nitrate RE was observed due both to the increase of influent loads and to the SRT reduction. At the end of phase II (average RE = 80%), nitrate RE reached the same value observed at the end of phase I (RE > 90%) and NO<sub>2</sub><sup>accumulated</sup>/NO<sub>3</sub><sup>reduced</sup> ratio decreased from 0.4 to 0.2. Same performances were maintained in the first 10 days of phase III. The absence of temperature regulation caused (from day 115) a reduction of denitrification performances due to temperature decrease. From day 140 nitrate RE higher than 90% and NO<sub>2</sub><sup>accumulated</sup>/NO<sub>3</sub><sup>reduced</sup> ratio lower than 0.15 were observed.

Sulphate constitutes more than 90% of the effluent sulphur compounds while more than 90% of the influent nitrogen was converted into nitrogen gas. In this context, Eq.3 was used to evaluate the effective impact of autotrophic denitrification process on sulfide removal.



Where N/S ratio is 0.36.

The effect of autotrophic denitrification on sulphide removal have been estimated as the difference between the experimental produced  $S_{sulphate}$  (Eq. 4) and the theoretical one (Eq. 5).

$$S_{sulphate, measured} = \frac{S - SO_4^{2-}}{S - SO_4^{2-} + S_0 + S - S_2O_3^{2-}} \quad \text{Eq. 4}$$

$$S_{sulphate, theoretical} = \frac{\Delta N}{(N/S)} \frac{S - SO_4^{2-}}{S_{tot}} \quad \text{Eq. 5}$$

A ratio between  $\Delta S_{tot, measured} / \Delta S_{tot, theoretical}$  equal to 1.06 and 0.94 resulted in steady state conditions in phase II and III, respectively.

## R2 performance evaluation

Figure 3 shows that, when nitrite alone was used as electron donor (R2) and steady state conditions are achieved, denitrification reached up to 80% of nitrite removal. In this conditions, thiosulphate partially accumulated in R2.

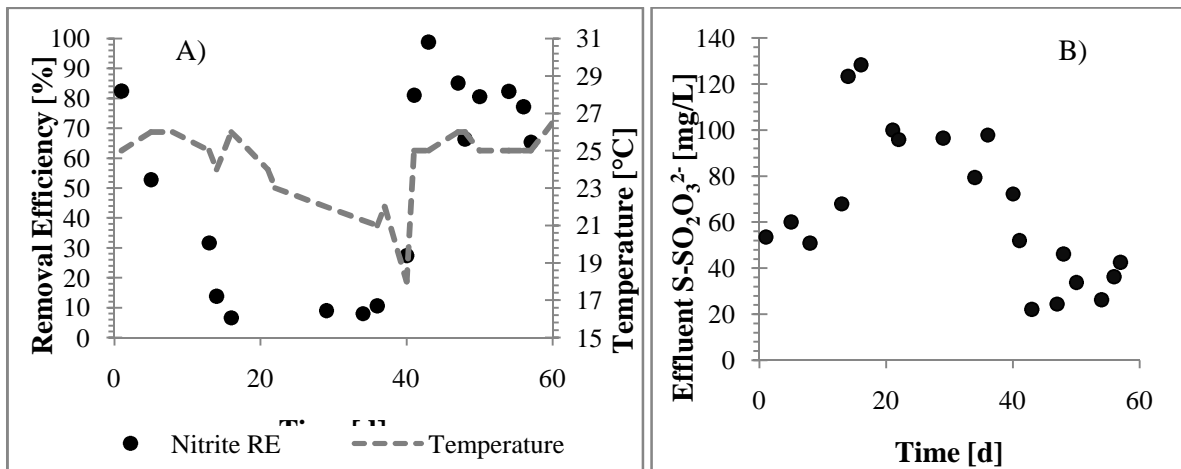
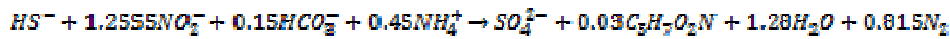


Figure 3 – Nitrite RE (A) and effluent thiosulphate (B) in R2

Autotrophic denitrification is highly influenced by temperature decrease: N-NO<sub>2</sub><sup>-</sup> RE decreases and the increase of CODsol indicates an increase in thiosulphate concentration. Thiosulphate production cause a reduction of sulphate in the effluent and an increase of the difference between the sulphate theoretically produced from the oxidation of the total influent sulphide and the measured one. On these basis, it is possible to hypothesize that sulphide oxidation to elemental sulphur and/or thiosulphate take place with kinetics significantly higher than oxidation of thiosulphate to sulphate; similarly, the reduction of nitrate to nitrite proceeds at higher transformation rates than denitrification. Sulphur compounds both in the influent and in the effluent of R2 referred to steady state (from day 45) are reported in Table 2.

The effect of autotrophic denitrification on sulphide removal have been estimated as reported for R1 using Eq. 6 (N/S = 0.55).



Eq. 6

A ratio between  $\Delta S_{\text{sulphate, measured}} / \Delta S_{\text{sulphate, theoretical}}$  equal to 0.95 resulted in steady state conditions.

## CONCLUSIONS

The results from this study indicate that both denitritation and denitrification with sulphide as electron donor are effective for the removal of sulphide in a wide range of operating conditions for what concern SRT, Temperature and S/N ratio. Sulphide removal efficiency was higher than 99% in all the tested conditions. Sulphide removal kinetics are extremely high, while nitrite tends to be partially accumulated as a function of SRT and T possibly due to slower kinetics of nitrite reduction respect to nitrate reduction to nitrite. The load of nitrogen present in tannery wastewater is compatible with the application of biogas biological treatment through autotrophic denitrification. In steady state conditions nitrogen removal efficiencies higher than 80% were obtained using nitrate or nitrite loads similar to those obtained by nitrification of the supernatant of an anaerobic digester treating vegetable tannery primary sludge.

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