Thermo-chemical pretreatment of a microbial biomass: influence of sodium hydroxide addition on solubilization and anaerobic biodegradability

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Abstract

The influence of sodium hydroxide addition during the thermo-chemical pretreatment of a microbial biomass has been evaluated in terms of particulate chemical oxygen demand (COD) solubilization and anaerobic biodegradability. Both COD solubilization and total solid elimination rates increased with the dose of NaOH added: COD solubilization reached 63%, and total solid elimination was 33% when 5 g Na/l were added. Additional sodium hydroxide addition did not increase COD solubilization further. The observed COD solubilization was due mainly to protein hydrolysis that was directly linked to pH variations. Heating emphasized these pH effects. The higher sodium hydroxide addition (26.1 g/l) led to 85% COD solubilization when heated to 140°C for 30 min instead of 53.2% which was at ambient temperature. Biodegradability and biotoxicity tests were run with pretreated samples. Both methane production and acetate degradation in the presence of pretreated samples were affected as 5 g NaOH/l or more were added. Sodium cation was first suspected to account for the limitations observed. Tests run at pH = 12 with other alkali agents (KOH, $Mg(OH)_2$, or $Ca(OH)_2$), led to the same conclusion: COD solubilization was enhanced but the biodegradability performances were limited. Additional tests run with acetate and increasing NaCl concentrations asserted that sodium cation was not at the origin of the limitations that were attributed to OH^- additions: refractory compounds were formed as a consequence of pH modifications. © 1999 Elsevier Science Inc. All rights reserved.

Keywords: Solubilization; Sodium hydroxide; Thermo-chemical pretreatment; Anaerobic biodegradability

1. Introduction

Thermo-chemical pretreatment can be used to solubilize organic matter before biological treatment when complex substrates such as sludge are concerned [1–3]. The effect of such a pretreatment is to promote hydrolysis and to split complex polymers into smaller molecules [4–6]. Many studies have focused on the determination of pretreatment conditions for improving solubilization and anaerobic biodegradability of waste activated sludge [1–4]. Optimal sludge pretreatment conditions reported are around 170°C for 30 to 60 min. They lead to important Chemical Oxygen Demand (COD) solubilization. However, some pretreatment conditions enhance the formation of complex molecules which are difficult to degrade, and as a consequence, optimal conditions defined for COD solubilization often differ from those used for optimal biodegradability achievement.

Some authors have combined chemical addition and thermal treatment. Alkaline treatment has been used to solubilize various substrates such as lignocellulosic materials [7–9] or waste activated sludge [10,11]. It allows significant solubilization and improves biodegradability performances. When it is combined with thermal treatment [2,4,9,12], it also leads to important solubilization. However, results tend to underline that extreme pH conditions are not compatible with anaerobic biological treatment. Patel et al. [9] have suggested that the biodegradability limitations observed for pretreated water hyacinth (120°C, pH = 13) could be a consequence of the sodium concentration. The sodium cation is known to be inhibitory to methanogenic flora [13–15]. Stuckey et al. [4] supported the view that extreme pretreatment conditions enhance the for-

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Table 1
Substrate characterization

Total COD (g/l)	56.2 ± 2.2
Soluble COD (g/l)	11.1 ± 0.8
TS (g/l)	30.6 ± 2.6
Total proteins (g/l)	20.7 ± 2.4
Soluble proteins (g/l)	2.8 ± 0.3
Total carbohydrates (g/l)	7.8 ± 1.1
Soluble carbohydrates (g/l)	1.8 ± 0.1
PH	4.7

mation of complex molecules through intramolecular reactions as Maillard reactions.

While studying the influence of pretreatment conditions of a microbial biomass on COD solubilization and anaerobic biodegradability, Penaud et al. [16] concluded that the optimal conditions for COD solubilization were 140°C and pH = 12 for 30 min. Under these pretreatment conditions, 71% COD solubilization was achieved. pH was the most important pretreatment parameter as regarded COD solubilization. Important limitations were observed during biodegradability tests. The hypothesis proposed were: 1) that some inhibitory molecules had been released; 2) that some intramolecular reactions had been induced leading to the formation of refractory compounds; 3) that other molecules as sodium cation had affected biodegradability performances.

The purpose of this study was to investigate the influence of sodium hydroxide on COD solubilization and anaerobic biodegradability of a microbial biomass with or without heating. Attention was focused on the separation of Na^+ and OH^- effects in order to identify the origin of the biodegradability limitations.

2. Materials and methods

2.1. Substrate characterization

The effluent was a spent microbial biomass obtained from an industrial plant. It consisted of micro-organisms and nutritive components. Table 1 shows the substrate composition.

2.2. Pretreatment conditions

2.2.1. Alkali agents addition
NaOH was added to 600 ml of substrate to reach concentrations ranging from 0 to 26.1 g NaOH/l. Additional tests were run at pH = 12 with various alkaline agents: NaOH, KOH, $Mg(OH)_2$, $Ca(OH)_2$.

2.2.2. Thermal treatment
300 ml of each sample were kept at ambient temperature, and 300 ml were heated at 140°C for 30 min. Each heating run was conducted in mechanically stirred autoclaves (working volume = 300 ml). The temperature was controlled via a PID regulation. The increase of temperature took 20 to 40 min, depending upon the temperature expected. Cooling time was 2 h.

2.3. Solubilization evaluation

Particulate organic matter solubilization was evaluated through COD solubilization and total solids reduction:

- COD solubilization = 100 (solubleCOD_f/total-COD_f) (*in* %), with;
- soluble COD_f: soluble COD measured after pretreatment
- total COD_f: total COD measured after pretreatment
- Total solids reduction = 100 (TS_f/TS_i) (*in* %), with;
- TS_f: total solids measured after pretreatment
- TS_i: initial total solids

2.4. Biodegradability and biotoxicity tests

Biodegradability and biotoxicity tests of pretreated samples were performed in 120-ml serum bottle at 37°C. Each test was run in duplicate.

2.4.1. Biodegradability tests
Methanogenic biodegradability tests were carried out by monitoring the gas production of a methanogenic sludge that had been acclimated to a synthetic solution consisting of glucose and VFA as carbon sources. A control test used acetate as a substrate. The initial loading of 2 g COD/l was used to feed each batch test. Methanogenic biodegradability was expressed as % biodegradability which was defined as follows: 100 * $V_{gas}/V_{theoretical}$ (V_{gas}: net volume of gas produced, $V_{theoretical}$: theoretical volume of biogas expected. This corresponds to the initial COD loading and assumes that 1 g COD can generate 600 ml of biogas).

2.4.2. Biotoxicity tests
During biotoxicity tests, the initial loading consisted of 2 g of COD/l of pretreated sample and of 1 g of COD/l of acetate. Gas production and acetate degradation rates were monitored. Results were expressed as % acetate degradation defined as follows: 100 * (C_2f-C_2i)/C_2i (C_2f: final acetate concentration (g/l), C_2i: initial acetate concentration (g/l)).

Additional biotoxicity tests were run with acetate and various NaCl concentrations calculated in order to have the same Na^+ concentrations as in the NaOH addition tests: 0; 0.66; 1.99; 2.65; 3.98; 5.30; 6.64; 8; 10; and 15 g Na/l. Other tests were realized by adding KCl, $Mg(Cl)_2$, and $Ca(Cl)_2$ in order to have the same Cl concentration as in the test realized by adding NaCl when Na concentration was corresponding to Na concentration obtained by adding NaOH to reach pH = 12.
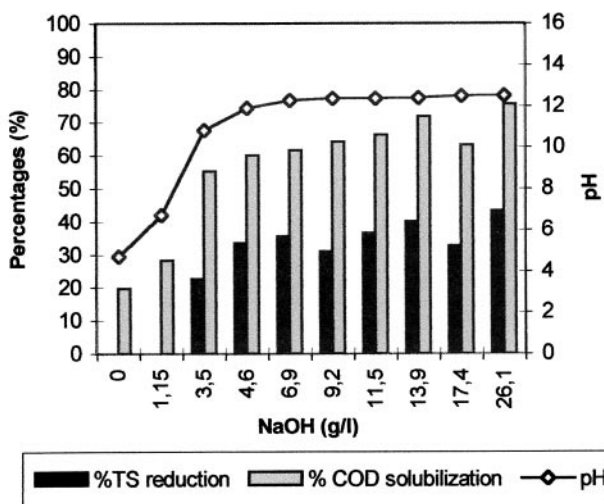
Fig. 1. Influence on NaOH addition on COD solubilization (ambient temperature).

Fig. 2. Influence of NaOH addition on COD solubilization when heating at 140°C for 30 min.

2.5. Analytical measurements

COD was measured by the potassium dichromate-ferrous ammonium sulfate method in accordance with standard methods [17]. The soluble COD was measured after centrifugation at 28 000 g for 15 min. Proteins were assessed by Lowry's method using Bovin Serum Albumin as a standard [18]. Total protein determination required a preliminary solubilization step with NaOH 1N at 100°C. The carbohydrates content of samples was estimated using Anthrone-sulfuric acid method with glucose as the standard [19]. Total solids (TS) were determined according to Standard Methods [17]. Volatile Fatty Acids (VFA) analyses were assayed by gas chromatography with nitrogen as carrier gas (splitless injector at 250°C, Econocap FFAP column, oven temperature: cycles from 80 to 120°C) with a flame ionization detector (Chrompack CP9000) coupled with an integrator (Shimadzu CR3A). Gas was analyzed by gas chromatography with Shimadzu GC8A apparatus using argon carrier with a catharometer detector (injector temperature: 100°C, oven temperature: 35°C). The chromatograph was coupled to a Shimadzu CR5A integrator. N-NH$_4$ measurements were done by the titrimetric method after distillation using a Büchi 320 apparatus [20].

3. Results and discussion

3.1. Influence of pretreatment conditions on solubilization of particulate COD

3.1.1. Influence of NaOH addition at ambient temperature

The influence of sodium hydroxide addition on COD solubilization and total solids reduction rates was evaluated. Results are reported in Fig. 1.
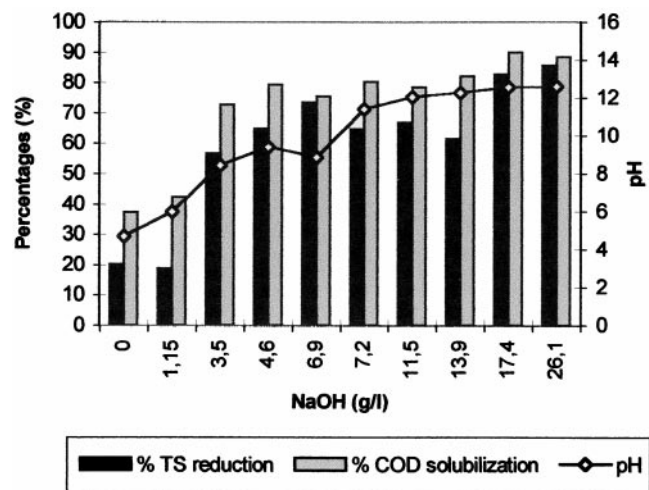
COD solubilization and total solids reduction increased as the dose of alkali increased, the COD solubilization reached 63%, and total solid elimination reached 33% when 4.6 g of NaOH/l were added. From 4.6 g of NaOH/l to 26.1 g of NaOH/l, a slower COD solubilization increase was observed (Fig. 1). COD solubilization evolution was linked to pH variations: important pH variations were leading to important COD solubilization. This COD solubilization was the consequence of protein solubilization. Soluble proteins were equivalent to 4.1 g of COD/l when no sodium hydroxide was added and 33.4 g of COD/l when 26.1 g of NaOH/l were used. Also N-NH$_4$ was released as a consequence of this protein solubilization; 95.2 mg of N-NH$_4$/l when no NaOH was added and 268.8 mg N-NH$_4$/l with 26.1 g of NaOH/l.

These results are in agreement with previous results reported from literature that adding 1.2 g of NaOH/l to waste activated sludge led to 45% solubilization of the particulate COD [10]. 15% volatile solids solubilization was achieved during combined sludge treatment because of the addition of 4.5 g of NaOH/l [12]. The COD solubilization obtained when alkali agents are added has been reported to be the consequence of various reactions such as saponification of uronic acids and acetyl esters, reactions occurring with free carboxylic groups, reactions of neutralization of various acids formed from immediate degradation of particular material [8].

3.1.2. Influence of NaOH addition when heating at 140°C for 30 min

The same concentrations of sodium hydroxide were added, and samples were heated for 30 min at 140°C. The same rates (total solids reduction and COD solubilization) were used to evaluate solubilization performances. Results are reported in Fig. 2.

For samples pretreated for 30 min at 140°C and pH = 12, COD solubilization was related to pH variations as it was when pretreatment was done at ambient temperature (Fig.

Table 2
Influence of various alkali agents addition on COD solubilization

Alkali agents (pH = 12)	Ambient temperature % COD solubilization	140°C % COD solubilization
NaOH	60.4	71.6
KOH	58.2	83.7
Mg(OH)$_2$	29.1	55.6
Ca(OH)$_2$	30.7	51.1

2). When 0 to 4.6 g of NaOH/l was added, the pH increased from 4 to 10 and COD solubilization increased from 37 to 80%. When more than 4.6 g of NaOH/l was added, the pH increased from 10 to 12 and COD solubilization slowly increased. Protein solubilization was observed, and as a consequence, N-NH$_4$ was released: 655 mg of N-NH$_4$/l when 26.1 g of NaOH/l was added. When comparing COD solubilization obtained when adding NaOH at ambient temperature or at 140°C, it can be concluded that heating emphasized the pH effects. For example, when no sodium hydroxide was added, 19.6% COD solubilization was achieved at ambient temperature and 37% at 140°C. When 26.1 g of NaOH/l was added, 75.4% COD solubilization were obtained at ambient temperature and 85.1% at 140°C.

Stuckey et al. [4] improved waste activated sludge solubilization by thermochemical pretreatment. They obtained 55% COD solubilization when pretreating at 175°C for 30 min and at pH = 12 instead of 48% COD solubilization at 175°C for 30 min without any pH adjustment. While pretreating combined sludge, Tanaka et al. [12] observed the positive influence of the temperature on alkaline solubilization: 15% of the volatile solid was solubilized by adding 4.5 g of NaOH/l without heating and 70% by adding 2.25 g of NaOH/l when heating at 130°C for 5 min.

3.1.3. Influence of the nature of the alkali

Additional pretreatments were done at pH = 12 with various alkaline agents NaOH, KOH, Mg(OH)$_2$, Ca(OH)$_2$. Tests were conducted at ambient temperature or at 140°C. COD solubilization performances are presented in Table 2.

Monobasic agents led to better solubilization percentages than dibasic ones probably because dibasic alkali agents were only partially dissolved. At ambient temperature, NaOH addition led to 60.4% COD solubilization and KOH addition to 58.2%. However, at 140°C, KOH addition led to 83.7% COD solubilization and NaOH addition to 71.6%. COD solubilization obtained with dibasic agents did not exceed 56% (Table 2). Some authors have previously reported that Ca(OH)$_2$ was less efficient than NaOH. Stuckey et al. [4] achieved 40% COD solubilization when Ca(OH)$_2$ was added (during waste activated sludge pretreatment at 175°C, pH = 12 for 30 min) rather than NaOH which led to 55% COD solubilization. During low-level alkaline solubilization of waste activated sludge, Rajan et al. [11] obtained
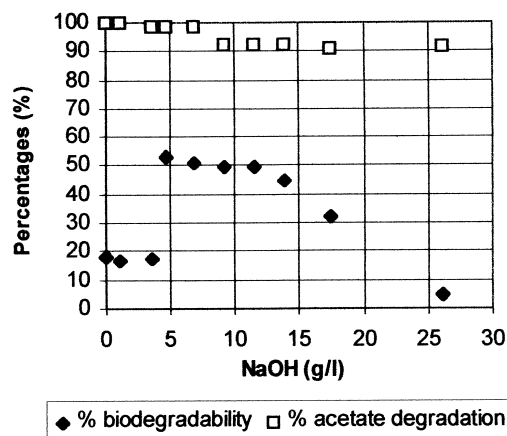

Fig. 3. Influence of NaOH addition on biodegradability and biotoxicity of pretreated samples (ambient temperature).

16 to 44% of particular COD hydrolysis when adding 10 to 30 mEq NaOH/l and only 14 to 18% when adding 20 to 40 mEq Ca(OH)$_2$/l.

3.2. Influence of pretreatment conditions on biodegradability and biotoxicity performances

3.2.1. Substrate pretreated with NaOH at ambient temperature

Fig. 3 presents results obtained during biodegradability tests (expressed as % biodegradability) and during biotoxicity tests (expressed as % acetate degradation) for samples pretreated at different sodium hydroxide concentrations at ambient temperature.

When less than 4 g of NaOH/l was added, biodegradability rates of pretreated substrates remained under 17%. When 4 to 10 g of NaOH/l was added, biodegradability rates reached 50%. However, when sodium concentrations exceeded 10 g of NaOH/l, biodegradability performances decreased (Fig. 3). Biotoxicity tests showed that the degradation of acetate obtained in the presence of pretreated samples began to drop from 8 g of NaOH/l where 92% of the fed acetate was degraded. However the level of acetate degradation inhibition did not increase with further NaOH concentration increase.

When the sodium hydroxide concentration was low, biogas production was low because organic matter was still particulate, and the rate-limiting step in this case was hydrolysis [21]. When high sodium hydroxide concentrations were used (26.1 g of NaOH/l), biogas production was still low. Most of the available COD was soluble. Hydrolysis of organic matter was no more than the rate limiting step. The problem was different as the limitation was caused by the solubilized molecules. However, acetate degradation was only affected slightly when such a sodium concentration was added since 92% of the loaded acetate was degraded.
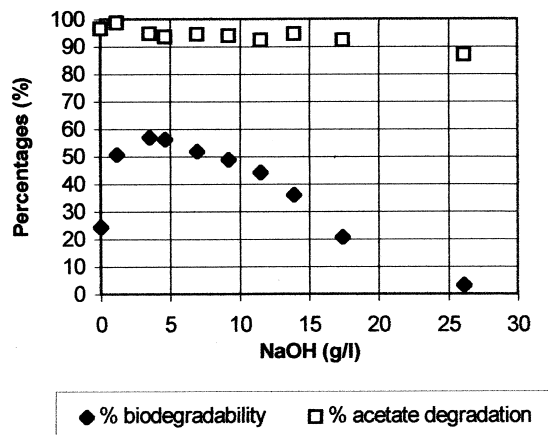
Fig. 4. Influence of NaOH addition on biodegradability and biotoxicity of pretreated samples (140°C for 30 min).

3.2.2. Substrates pretreated with NaOH when heating at 140°C for 30 min

Results obtained (expressed as % biodegradability obtained during methanogenic batch tests and as % acetate degradation obtained during inhibition tests) are shown in Fig. 4.

The highest biodegradability rates were obtained when added sodium concentration was around 4–5 g of NaOH/l. The increase in biogas production observed between 0 and 5 g of Na/l added was significant, and it raised the biodegradability rates from 22 to 58%. Then, the biodegradability rates progressively decreased with NaOH increase. When 26.1 g of NaOH/l was added, the biodegradability rate was below 5% (Fig. 4). Inhibition tests revealed that acetate degradation was around 90% for all samples pretreated with more than 4 g of NaOH/l.

Table 3 compares biodegradability rates obtained with substrates treated with NaOH at ambient temperature and at 140°C.

When less than 5 g of NaOH/l was added, biodegradability rates were higher for the heated samples. This can be explained by the higher solubilization obtained when samples had been heated. However, when more than 5 g of NaOH/l was added, biodegradability rates were low for both heated and non heated samples. When 26.1 g of NaOH/l was added, biodegradability rates were below 10% (Table 3). Biodegradability performances were lower when samples had been heated even if solubilization was more important in this condition.

Table 3
Comparison of biodegradability rates of heated and nonheated samples

Sodium added	Non-heated samples	Heated samples
0 g NaOH/l	17%	22%
5 g NaOH/l	45%	60%
13.9 g NaOH/l	44.7%	36%
17.4 g NaOH/l	32.1%	20.6%
26.1 g NaOH/l	5%	3.2%

Table 4
Influence of cations addition on acetate degradation

NaCl added (g/l)	% Acetate degradation
0	100
0.66	100
1.99	100
2.65	98.9
3.98	100
5.30	100
6.64	98.6
8.00	92.9
10.0	95.8
15.0	88.6
KCl added	96.7
Mg(Cl)$_2$ added	95.8
Ca(Cl)$_2$ added	100.7

During the thermochemical pretreatment of waste activated sludge, Stuckey et al. [4] observed that NaOH addition improved the conversion of total COD to biogas, whereas the conversion of soluble COD to biogas was not improved. Haug et al. [1] reported that alkali addition adversely affected digester performances soon after feeding began and that no acclimatization occurred. NaOH addition was able to enhance COD solubilization, but solubilized COD conversion was not improved. Heating emphasized alkaline solubilization but did not improve biodegradability rates.

3.2.3. Influence of the nature of the alkali

When considering biodegradability, the performances obtained when the pretreatment was done with various alkaline agents, no differences were observed with NaOH, KOH, Mg(OH)$_2$, or Ca(OH)$_2$. It suggested that the low biodegradability performances observed were not a consequence of sodium cations concentration. This was also confirmed by acetate biodegradability tests run with increasing sodium cation concentrations (0 to 15 g of Na/l added as NaCl). Results are reported in Table 4.

When 0 to 8 g of Na/l was added, almost complete acetate degradation was observed, indicating that no inhibition occurred. Beyond 8 g of Na/l, acetate degradation began to be affected: only 88.6% of the loaded acetate was degraded when 15 g of Na/l was added (Table 4). Control tests run with KCl, CaCl$_2$, and MgCl$_2$ led to more than 95% acetate degradation what asserted that Cl anions had no influence on results obtained.

The conclusion proposed was that the presence of sodium cation did not cause any limitation. Low biodegradability performances were attributed to the nature of the solubilized molecules. Analogous limitation problems have already been reported for samples pretreated at extreme temperature [2,4] or when concerning thermo-chemical pretreatment, under extreme pH conditions [9]. Too severe pretreatment conditions enhance the formation of refractory compounds. The hypothesis, proposed to explain the refrac-

tory nature of the molecules formed, is that some intermolecular reactions occur between solubilized compounds which lead to the formation of complex substances. Such reactions are more commonly named Maillard reactions and are frequently observed in the food industry [22–25]. Colored molecules, melanoidins, are formed. Such colored compounds are reported to be complex and very difficult to degrade even by rumen micro-organisms [26,27].

4. Conclusion

Alkali treatment allowed an enhanced solubilization which was even more pronounced when heating was applied. When more than 5 g of NaOH/l was added, 75–80% COD solubilization was achieved when heating at 140°C for 30 min instead of 65% at ambient temperature. Nevertheless, too high NaOH addition (more than 5 g of NaOH/l) did not lead to further significant increase of COD solubilization.

When more than 5 g of NaOH/l was added, biodegradability of heated and nonheated samples was low. This was not a consequence of sodium cation concentrations but had to be related to hydroxide addition. Hydroxide addition caused the pH increase that was responsible for the COD solubilization but also led to the formation of refractory compounds. For example, colored molecules were formed through reactions commonly named Maillard reactions.

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