(21) ALKALINE, ACID AND THERMAL SOLUBILIZATION FOR MINIMIZATION OF WASTE SLUDGE
発生汚泥量の減容のためのアルカリ・酸・熱処理による汚泥可溶化

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Abstract; In the activated sludge plants, the excess sludge is currently processed at considerable costs, and, the reduction of it is a major concern. Reducing weight, volume and organic content has been conventionally achieved through biological digestion techniques, but these require long and uneconomical period to reach stabilization. In this study, the efficiencies of alkaline, acid and thermal methods in minimizing the sludge have been investigated and compared with the traditional methods of treating the excess sludge. Maximum possible MLSS reduction was 68% when the alkaline treated sludge was autoclaved at 121 °C for 30 mins. The higher the pH, the higher the MLSS reduction with maximum of 64% at pH of 12.5 after 24 hours. The lower the pH, higher the MLSS reduction with maximum of 28% at pH 2.5 at room temperature. Alkaline solubilization yielded higher reduction of MLSS compared to acid solubilization due to the reasons that alkaline solubilization solubilized most of the protein in MLSS whereas acid solubilized carbohydrate portion of the sludge and that MLSS contained around 10 to 15% carbohydrate and 35 to 40 % of protein COD. The higher the temperature, the higher is the soluble COD yield. And thermal hydrolysis at 121 °C and 1 bar, yielded solubilization of 44% and 41% of protein and carbohydrate COD respectively.
Keywords; Sludge solubilization; alkaline treatment; acid treatment; sludge reduction; COD yield.

1. Introduction

In the activated sludge plants, the excess sludge is currently processed at considerable cost. The total cost of excess sludge treatment and disposal can represent up to 60% of the total operating cost as reported by Canales *et al.*, (1994). Therefore the reduction of the excess sludge has become a major concern. Reducing the organic content of the sludge has been conventionally achieved through biological digestion techniques, but these require long and uneconomical periods to reach stabilization. Since the hydrolysis of pariculates to soluble hydrolysis products is the rate limiting step in the acid phase of anaerobic digestion (Eastman *et al.*, 1981), and the solids reduction rate is slowed by even small addition of waste activated sludge because the potential substrates are membrane enclosed within viable cells (Han *et al.*, 1997), sludge solubilization by thermal and mechanical pretreatment has been studied by Dohanyos *et al.*, (1997) in order to intensify the anaerobic methane fermentation of sludges using cell lysate as a stimulating agent. The most recent technology reported by Yasui *et al.*, (1996) to eliminate the excess sludge in the activated sludge process is ozonation in which no excess sludge was needed to be withdrawn when COD loading was less than 550 kg/d of BOD loading. Clark (1997) who investigated thermophilic aerobic digestion for sludge reduction reported mean retention time of at least 7 days digestion at 55 °C is necessary for efficient sludge reduction. Kyung-Yub *et al.*, (1997) who investigated the possibilities of rupturing the waste activated sludge by mechanical homogenization and smashing under pressurized conditions, concluded that anaerobic digestion efficiency can be greatly increased by mechanical pretreatment of waste activated sludge. Apart from the above mentioned technologies, alkaline, acid and thermal treatment of sludge can also solubilize the excess sludge finally disposed. The present study focuses on the possibilities of extracting the carbon sources from the excess sludge by chemical and thermal methods, and feeding them as carbon sources for denitrification purpose wherever they are needed, instead of using the traditional external carbon sources such as methanol or acetate.

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V. Aravinthan *et al.*, (1997) proposed a system in which excess sludge from an activated sludge reactor could be solubilized with alkali and used as a viable carbon source for denitrification. However, the sludge reduction techniques using chemical and thermal methods and the usage of extracted carbon sources for denitrification by different methods of solubilization, have not, since then, been thoroughly and comprehensively investigated.

Therefore, the objectives of the present study are to investigate the possibilities of sludge reducing techniques by means of alkaline, acid and thermal hydrolysis, and to compare the efficiency of sludge solubilization and reduction of MLSS with that of biological methods.

2. Materials and Methods

2.1 Batch experiments on sludge solubilization

Experiments were conducted to find out the sludge solubilization in pH ranges from 2 to 4.5 for acid environment using HCl and from 9 to 12.5 for alkaline environment using NaOH. The sludge amounting to 1 l having pH between 7 to 7.5 was withdrawn from a sequencing batch fluidized bed bioreactor, which is described in details below. pH was adjusted from 2 to 4.5 and 9 to 12.5 and the sludge was kept closed in stirred conditions in pyrex vessel of 1 l. Samples were withdrawn and filtered by 0.45 μm whatman glassfiber filters, to measure soluble COD and soluble nitrogen for a period of 6 hours in suitable interval and after a day. The initial and final MLSS concentrations as well as total COD and total nitrogen were also measured. Similar experiments were conducted in different temperatures with and without the addition of acid and alkali.

Sludge solubilization in the autoclave was also investigated under acid, alkali and neutral environment, at temperature of 121 °C and pressure of 1 bar. Sludge samples amounting to 1 l were solubilized in the autoclave for 30 mins in a pyrex vessel in closed conditions. Samples for which pH was adjusted, were stirred for 1 hour prior to autoclaving. Total time taken for the solubilization process can be considered to be 4 hours including the time taken for the preparation and cooling after autoclaving. In repeated experiments, in addition to the above parameters, total and soluble proteins and carbohydrates were also measured at the beginning and the end.

2.2 Source of sludge for batch experiments

Sludge was withdrawn for solubilization from the sequencing batch fluidized bed bioreactor. The total working volume of the reactor was 14.25 l. After the effluent decanting, the volume retained was 10 l. The contents of the synthetic substrate are shown in Table 1. The organics and other micro-nutrients needed for bacterial synthesis were provided by acetate, peptone and yeast extract. Nitrogen and phosphorus were supplied as NH_4Cl and KH_2PO_4 and alkalinity for autotrophic bacteria as $NaHCO_3$. Everyday, four cycles of 6 hours were performed. Each cycle consisted of a feeding phase (15 min), anoxic phase (1 hour), oxic phase (4 hour), sedimentation phase (30 min) and a decanting phase (15 min). After the reactor has reached the steady state conditions, the sludge from the reactor has been used for batch experimental work. Sludge retention time was maintained to be 12 days by withdrawing sludge from aerobic phase.

Table 1 Contents of Synthetic Wastewater

Contents	mg/l
Glucose	50
Yeast extract	50
Peptone	50
Acetate	50
NH_4Cl (as N)	30
KH_2PO_4	14
K_2HPO_4	14
KCl	20
NaCl	10
$MgSO_4$	6
$NaHCO_3$	240

2.3 Analytical methods

MLSS, total COD, soluble COD, soluble total nitrogen and total nitrogen were all measured in accordance with the procedures demonstrated in the Standard Methods (AWWA, 1992). Colloidal COD of MLSS was also measured. The sample after solubilization was centrifuged at 4000 rpm for 15 mins, and the sample of the supernatant was measured for COD that contains both soluble and colloidal COD. The supernatant was then filtered by 0.45 μm whatman glassfiber filter to measure the soluble COD. The difference between the two would give the concentration of colloidal COD. Protein was measured using the method proposed by Lowry *et al.*, (1951) using bovine serum albumin as a standard solution. Carbohydrate was measured using the method proposed by Duboius *et al.*, (1956) that makes use of 5% phenol and 97% H_2SO_4 and using glucose as a standard solution. All protein and carbohydrate concentrations were expressed in terms of COD.

3. Results

3.1 Alkaline and acid sludge solubilization using NaOH and HCl at different pH

a) Suspended matter

Alkaline and acid solubilization were carried out using the chemicals NaOH and HCl to the desired pH, and the reaction was terminated by neutralization after 6 hours. Sludge mixed liquor, after solubilization, is divided into liquid phase and solid phase: the former contains both soluble and colloidal parts, and the latter remains unsoluble. Suspended matter in this discussion was measured in terms of COD, therefore the dissolved solids added by way of acid and alkali will not affect suspended matter measurements. Fig. 1 shows the different portions of MLSS such as insoluble, colloidal and soluble part that were produced in the solubilization process after 24 hours. Suspended matter destructions at the end of 24 hours when pH was 12.5, 12.0, 10.0 and 9.0 were 64, 62, 33 and 8% respectively. The measured suspended matter, in this discussion, contains only the insoluble part that settles in the centrifuge at 4000 rpm for 15 min. The supernatant after centrifugation was also measured for the (colloidal+soluble) COD. The amount of colloidal COD was highest when pH was 12.0. The difference in the solubilization between pH 12.0 and 12.5 is the conversion of the colloidal COD to the soluble one when the pH was 12.5. Acid solubilization reduced lesser amount of sludge than alkaline one. Suspended matter destructions at the end of 24 hours were 28, 21 and 14% when pH was 2.0, 3.0 and 4.5 respectively.

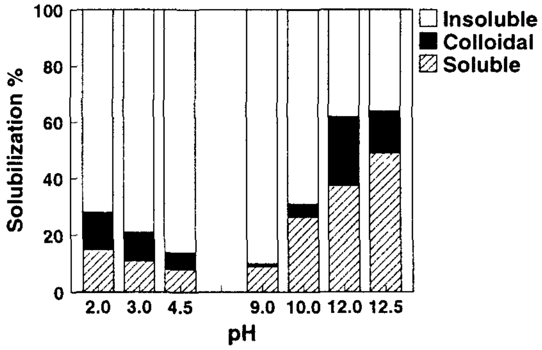
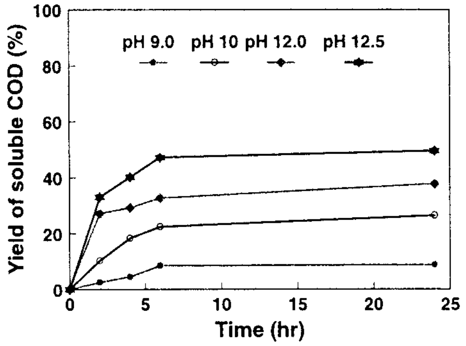


Fig. 1 MLSS solubilization at different pH

b) COD and total nitrogen

During the solubilization process, flocs are first dispersed to finer particles like colloids and then cell membrane is ruptured, as a result, carbon, nitrogen and other elements are released into bulk solution. It is intended to obtain maximum possible carbon source with highest reduction of solids from the solubilization process. So the yields have been defined in terms of soluble COD and total N as follows:

Yield C= (Final soluble COD - Initial soluble COD) / (Initial total COD- Initial soluble COD)
Yield N= (Final soluble TN - Initial soluble TN) / (Total TN- Initial soluble TN)



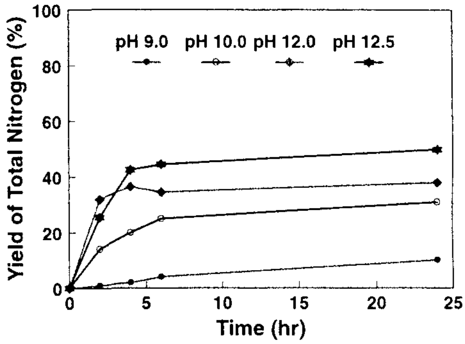


Fig. 2 Soluble COD yield with time

Fig. 3 Total nitrogen yield with time

Solid solubilization was found to occur at an accelerated speed till 6 hours and at a very slow pace after that, so the degree of solubilization after 6 hours was not considerable as shown in Fig. 2. The reaction could be considered as purely chemical where the reaction time was about 6 hours. The temperature was found to increase from 20 to 28 °C.

The higher the pH, the higher the yield with maximum of 47% at pH of 12.5 after 6 hours. The results were reproducible for MLSS concentration in the range 2 and 4 g/l and at the average temperature of 25 °C. Total nitrogen present in the system was also increasing, giving nearly same yields as those of COD as shown in Fig. 3. The presence of ammonia, nitrite and nitrate nitrogen were negligible. Almost all the total soluble nitrogen is in the form of organic nitrogen.

Similarly, the acid solubilization also required the detention time of 6 hours for complete solubilization and longer time did not alter the degree of solubilization. The lesser the pH, the higher the degree of solubilization. However, the degree of solubilization was higher for alkaline solubilization than acid one. The average COD yield reduction obtained at 6th hour was plotted against the pH. There was a drastic increment in COD yield in the case of alkalıne solubilization compared to the mild increment in the case of acid solubilization as shown in Fig. 4.

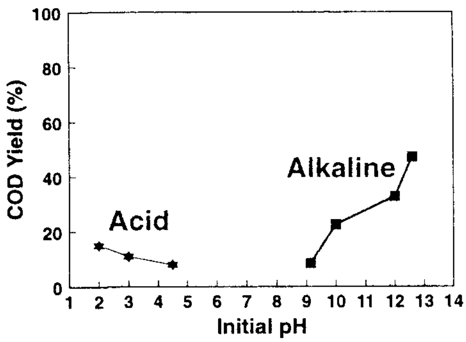


Fig. 4 Soluble COD yield at different pH at 6th hour

After the solubilization process, pH tends to come down by one fold in the case of alkaline solubilized substrate and went up by one fold in the case of acid solubilızed one. This is advantageous as neutralization requires lesser amount of acid or alkaline as the case may be.

3.2 Alkaline and acid sludge solubilization at elevated temperatures with constant pH

The alkaline and acid treatments given at room temperature successfully solubilized 64% and 28% of MLSS at pH 12.5 and 2.0 respectively. Since the chemical reaction could be accelerated by raising the temperature, series of solubilization experiments were conducted at 40°C and 60 °C after the pH of the sludge was raised and lowered to 12.5 and 2.5 respectively.

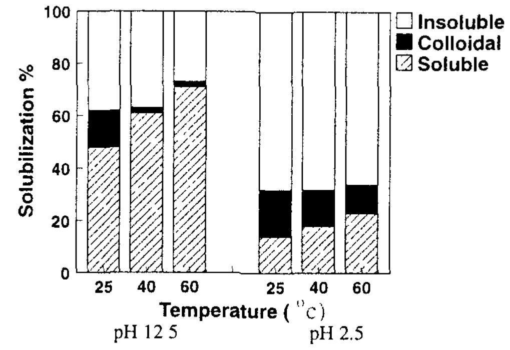


Fig. 5 MLSS solubilization with temperature at different pH

When the pH was 12.5, MLSS reductions were 62, 63 and 73 % at the temperatures 25, 40 and 60° C as shown in Fig. 5. The percentage of solubilization and corresponding COD yields were 48, 61 and 71 % for 25 °C, 40 °C and 60 °C respectively as shown in Fig. 6. In the room temperature, there was a great difference between the percentage reduction of MLSS and the produced soluble COD, due to higher presence of colloidal matters. However, in the higher temperatures, there was little colloidal COD indicating higher temperatures induce the conversion of colloidal matters also into soluble form in addition to reducing more MLSS as shown in Fig. 5. This explains why there was little difference in the MLSS reduction between that in room temperature and at 40 °C.

When the pH was 2.5 and the temperature was elevated, the yield of soluble COD increased but not to the same degree as in alkaline solubilization as could be seen in Fig. 7. The maximum degree of solubilization of 24% has been obtained at 60 °C, corresponding to 34% of MLSS reduction. There was little increase in the overall MLSS reduction in higher temperatures. Colloidal MLSS was reduced to more soluble forms in higher temperatures as shown in Fig. 5. The increment of MLSS reduction was higher for alkaline solubilization compared to acid one.

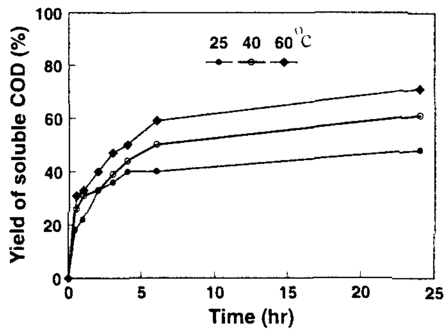


Fig. 6 Degree of solubilization with temperature at pH 12.5

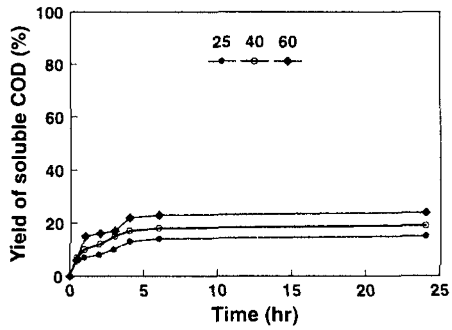


Fig. 7 Degree of solubilization with temperature at pH 2.5

3.3 Alkaline and acid sludge solubilization in the autoclave with constant pH

In these experiments, pH was adjusted to 3 and 11 for HCl and NaOH respectively. Protein and carbohydrate COD were measured for soluble part of the MLSS. Fig. 8 shows the degree of solubilization of different components of COD at different pH and temperature (room temperature, 60 °C and autoclave at 121 °C and 1 bar). Alkaline and acid solubilization in autoclave (121 °C and 1 bar) solubilized about 81 and 41 % of protein and 52 and 80% of carbohydrate in MLSS. Alkali added solubilized most of the protein in the sludge whereas acid acted upon the carbohydrate portion of the sludge. Since protein concentration (35 to 40%) in MLSS was higher than that of carbohydrate (10 to 15%), alkaline

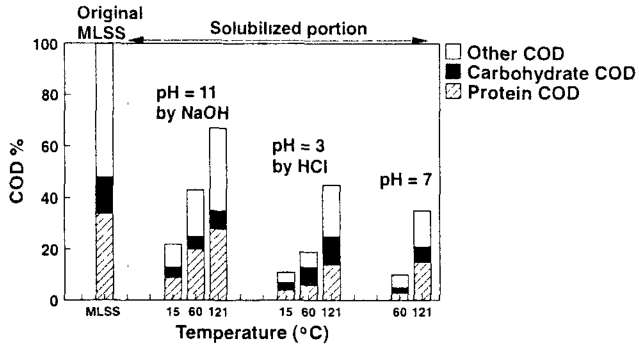


Fig. 8 Effect of temperature and pH on carbohydrate and protein solubilization

solubilization gave higher amount of solubilization. Thermal hydrolysis without addition of any chemicals also was effective in solubilizing 44 and 41% of protein and carbohydrate in MLSS. The advantage of having thermal hydrolysis is the solids liquid separation that is achieved automatically without the need for centrifugation.

4. Discussions

4.1 Rating the methods of solubilization

The MLSS reduction and corresponding COD yields were different when different methods of solubilization were applied. The highest MLSS reduction as well as maximum soluble COD yield were obtained for alkaline solubilized sample at pH 11 in the autoclave as shown in Fig. 9. The acid solubilized sludge hydrolysate at pH 3 in the autoclave as well as the alkaline solubilized samples at pH 11 at 60 °C and the alkaline solubilized sample at pH 12 in the room temperature gave nearly same COD yields. In terms of MLSS reduction and solubilization, alkaline solubilization with NaOH in the autoclave can be preferred compared to acid solubilization. And the thermal solubilization in the autoclave gave 10% lesser degree of solubilization compared to acid solubilization in the autoclave. The addition of the acid to enhance the sludge hydrolysis in the autoclave is not very efficient since the corresponding increase in sludge reduction was not very considerable compared to thermal hydrolysis. Now comparison can be done with thermally hydrolyzed sludge and alkaline autoclaved sludge hydrolysate. The main advantage of thermal treatment is the separation of solids and liquids that are automatically achieved without the need for centrifugation which is necessary in alkaline or acid treatment of sludge. In cases chemicals are not encouraged to be used in the treatment facilities, thermal treatment can be considered. The most appropriate method ultimately to be selected for application would be the

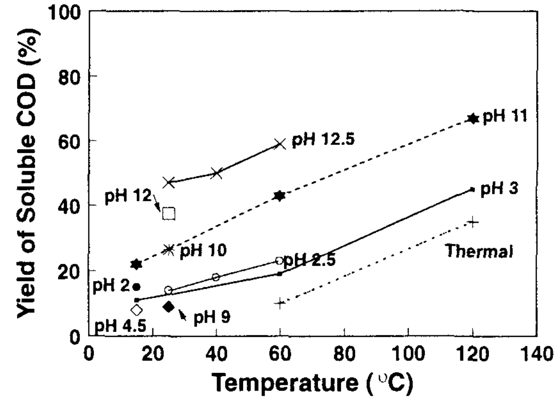


Fig. 9 Summary of degree of solubilization

one that gives highest sludge reduction, yields best biodegradability of the sludge hydrolysate and gives the least cost in terms of economical aspects.

4.2 Comparison with biological sludge reduction methods from literature

The biological digestion techniques can be either aerobic or anaerobic ones. Tran *et al.*, (1990) who studied aerobic digestion of primary and secondary sludges in airlift bioreactors at mesophilic temperatures have reported 33% Volatile Solids (VS) reduction in the pilot plant reactor at mesophilic temperature range between 31°C and 33 °C at HRT of 4 days as shown in Table 2.

Table 2 Review of VS reduction by biolgical digestion methods

Source	Published year	Temperature (°C)	Retention time / d	Method of treatment	Degree of VS reduction (%)
Tran	1,990	31-33	4-5	Aerobic Airlift Reactor	33
Oles	1,997	55-60 35-37	2-3 12-15	Anaerobic egg-shaped reactor	60
Pagilla	1,997	36	16	Anaerobic digestion (mechanically and gas mixed)	54-62
Li	1,997	36	30	Anaerobic digestion (Full scale egg-shaped reactor)	54.2
Peres	1,992	35-37	15	Mesophilic Anaerobic digestion	45-50

Oles *et al.*, (1997) who investigated the combined thermophilic and mesophilic digestion in egg-shaped reactors have reported the organic solids reduction of 60% compared to the 48% in the mesophilic conditions under a total of 17 days. Pagilla *et al.*, (1997) who investigated mesophilic anaerobic digestion with mechanically or gas mixed digestors have reported higher VS reduction (54 to 62%) compared to the conventional techniques. Li *et al.*, (1997) who has investigated ten full scale egg-shaped reactors have reported 54.2% VS reduction at mesophilic conditions at a retention time of 30 days.

New advanced methodologies have been adopted in order to increase the process efficiency of biological digestion techniques. Anaerobic digestion continues to be the dominant sludge stabilization process because of the energy conservation and recovery out of wastewater sludge. Aerobic digestion is not preferred due to higher power cost, associated with supplying the required oxygen and inability to recover the useful by-product such as methane. Most of the modern anaerobic digestion techniques aided by different configurations as well as type of mixings have improved the efficiency of VS reduction as shown in Table 2.

Maximum achievable sludge reduction was 68% when the sludge was autoclaved after adjusting the pH to 11. In terms of sludge reduction, both chemical and biological methods tend to give similar results. However, chemically and thermally assisted sludge reduction methods can reduce the sludge in a matter of 6 and 3 hours respectively, whereas the anaerobic digestion techniques require at least 15 days for such stabilization resulting in considerable space saving. Chemically assisted sludge reduction techniques can also eliminate the need to depend on the delicate biological operations needed for efficient anaerobic digestion and reduce the odor problems that are inevitable in the anaerobic digestion techniques.

4.3 Potential Utilization of the extracted carbon sources

The solubilization of these waste sludge, if achievable in the plant, then can be used as a carbon source for denitrification, (both in pre and post denitrification, instead of using traditional external carbon sources such as methanol and acetate) and also can reduce the final volume and weight of sludge discharged into the sludge treatment. V. Aravinthan *et al.*, (1997) has reported that alkaline solubilized sludge hydrolysate could be a viable carbon source. The initial denitrification rates of sludge hydrolysate and acetate were nearly equal, indicating the potential of sludge hydrolysate as a denitrification

Table 3 Review of hydrolysis reported in the literature

Source	Published year	Temperature (°C)	Detention time /d	Degree of solubilization (%)
Eastman Ferguson	1,981	35	2.75	24
Anderson	1,989	-	-	15-20
Karlsson	1,989	37	1	17.8
Jorgension	1,990	-	1	8-12
Jorgension	1,990	-	3	12-15
Jorgension	1,990	30	3	15

accelerator. However, the denitrification rate of sludge hydrolysate showed another lower rate after all easily degradable substrates were used up, whereas it remained the same until nitrate was depleted in the case of acetate, indicating the hydrolysis rate of slowly degradable carbon in the sludge hydrolysate to be the rate limiting step of the denitrification. Kristensen *et. al.* (1992) studied the usage of sludge hydrolysate from anaerobic reactor for denitrification. And Wang *et al.* (1995) concluded that alkaline pretreatment of waste activated sludge results in enhancement of its anaerobic digesting efficiency. In order to intensify the anaerobic methane fermentation of sludges, Dohanyos *et al.*, (1997), has solubilized the sludge by thermal and mechanical treatment and analyzed the use of cell lysate as a stimulating agent in reducing the sludge.

The soluble COD available in the biological hydrolysis methods are listed in Table 3. Biological hydrolysis is a process in which particulate organic matter is solubilized and fermented to volatile fatty acids, but not further to methane and corresponds to the acid phase of anaerobic degradation (Jorgensen, P. E., 1990). The main part of the solubilized COD from biological hydrolysis was made up of volatile fatty acids that can be easily taken up by the cells. Soluble COD in the biological reactors was found to be in the range of 10 to 15 % of the total COD in the influent sludge among which, 60 to 80%, was volatile fatty acids (Jorgensen, P. E, 1990).

Biological hydrolysis can give considerably higher denitrification rates than chemical hydrolysis as the characteristics of carbon sources are quite different; the former contains most of the fatty acids whereas the latter has complex cellular components. However, compared to the biological method of hydrolysis, alkaline and thermal methods gave 3 to 4 times higher degree of solubilization in considerably lesser retention time. The time taken for the biological hydrolysis with lesser degree of solubilization is 2 or 3 days whereas the chemical and thermal treatment can be terminated within 6 and 4 hours respectively.

4.4 Factors that can affect the degree of solubilization

Soublilization as well as the biodegradability of the suspended solids removed by the primary sedimentation may be higher than that of the excess sludge, the reason being that the carbon source that has been digested by the bacterial cells is extracted from the bacterial cells after breaking down their membranes whereas the suspended solids do not have their carbon source inside cells or membranes. Similarly, the floc size and the density of the bacterial cells may also affect the percentage of solubilization. The denser the sludge, the more difficult it might be to solubilize as the chemical or heat treatment should penetrate into colonies of cells. And another factor that can affect the degree of solubilization is the type of substrate and the wastewater treatment facilities in which the sewerage are treated. The synthetic wastewater prepared for the laboratory scale reactors and the actual sewerage that is treated in the wastewater treatment plants can alter the results. Therefore, it might be difficult to achieve similar results with different type of sludge. However, the study undertaken in the laboratory scale reactor with synthetic wastewater can give an indication that sludge reduction by alkaline, acid and thermal methods could be promising for application in the field.

5. Conclusions

The following conclusions can be arrived regarding the alkaline, acid and thermal methods for solubilization :

1) Maximum possible sludge reduction that could be achieved was 68% when the sludge was subjected to alkaline treatment at pH 11 and autoclaved for 30 mins at 121 °C and pressure of 1 bar, yielding nearly same soluble COD. The alkaline treatment in the room temperature gave 64% of MLSS reduction yielding 49% of soluble COD yield after 24 hours.

2) COD solubilization increased steadily until 6 hours, and the degree of MLSS reduction after 6 hours was not very considerable. The reaction can therefore be terminated at the end of 6 hours.

3) The higher the pH, the higher the MLSS reduction with maximum of 64% yielding 49% COD yield at pH of 12.5 after 24 hours. The lower the pH, the higher the MLSS reduction with maximum of 28% reduction yielding 15% yield of soluble COD at pH 2.5 at room temperature.

4) Alkaline solubilization yielded higher MLSS reduction compared to acid solubilization due to the reasons that alkaline solubilization solubilized most of the protein in MLSS whereas acid solubilized carbohydrate portion of the sludge and that MLSS contained around 10 to 15% carbohydrate and 35 to 40 % of protein COD.

5) The higher the temperature, the higher is the soluble COD yield. And thermal hydrolysis at 121 °C and 1 bar, yielded 44% and 41% of protein and carbohydrate COD respectively.

In order to consider the application of the method in the field, the possibilities of using the extracted carbon sources for different purposes such as to improve the denitrification potential in the wastewater treatment facilities or to increase the methane gas formation in the anaerobic digestion processes have to be explored. The effects of different solubilization methods on the biodegradability are also worth investigating.

References

Anderson, B. and Rosen, B. (1989) Upgrading for biological nitrogen removal -some full scale experiences from sweden. *Proc. of International Specialized Conference on Upgrading of Wastewater Treatment Plants*, Munich, 3-7, pp. 89-100.

Aravinthan,V., Komatsu, K., Takizawa, S., Ohgaki, S. and Otaki.M (1997) Denitrification of sludge hydrolysate by immobilized microorganisms, *Proc. of 6th IAWQ Asia-Pacific Regional Conf.*, Vol. 1, pp. 822-829.

Canales, A., Pareilleux, A., Luc Rols, J., Goma, G. and Huyaard. A (1994) Decreased sludge production strategy for domestic wastwater treatment, *Wat. Sci. Tech.*, Vol. 30, No.8, pp. 97-106.

Clark, P.B. (1997) Thermophilic Sludge Digestion For Sludge Pasteurization, A Review. *Proc. of 6th IAWQ Asia-Pacific Regional Conf.*, Vol. 1, pp. 477 - 486.

Dohanyos, M., Zabranska, J. and Jenicek, P (1997) Innovative technology for the improvement of the anaerobic methane fermentation, *Wat. Sci. Tech.*, Vol. 36, No.6-7, pp. 333-340.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. and Smith, F. (1956) Colorimetric method for determination of sugars and related substances, *Analyt. Chem.*, 28, pp. 350-356.

Eastman, J.A. and Ferguson, J.F. (1981) Solubilization of particulate organic carbon during the acid phase of anerobic digestion, *J. WPCF*, 53, pp. 352-366.

Han, Y., Sung, S. and Dague, R. (1997) Temperature-phased anaerobic digestion of wastewater sludges, *Wat. Sci. Tech.*, Vol. 36, pp. 367-374.

Jorgension P.E (1990) Biological Hydrolysis of Sludge From Primary Precipitation, *Chemical Water and Wastewater Treatment*, pp. 511-519.
Karlsson, I. and Smith, G. (1989) Pre-precipitation facilities nitrogen removal without tank expansion. *Proc. of International Specialized Conference on Upgrading of Wastewater Treatment Plants*, Munich, 3-7, pp. 81-88.
Kristensen, G.H., Jorgensoen. P.E., and Henze, M., (1992) Characterization of functional microorganism groups and substrate in activated sludge and wastewater by AUR NUR and OUR, *Wat. Sci. Tech*, Vol. 25, pp. 43-57.
Kyung-Yub.H., Eung-Bai, S. and Hong-Bok,C. (1997) A mechanical pretreatment of waste activated sludge for improvement of anaerobic digestion system, *Proc. of 6th IAWQ Asia-Pacific Regional Conf.*, Vol.1, pp. 503-508.
Li, Y.Y., Mizuno,O., Miyahara,T., Noike,T., and Katsumata. K (1997) Ecological analysis of the bacterial system in a full-Scale egg-shaped digester treating sewage sludge, *Wat. Sci. Tech.*, Vol. 36, pp. 471-478.
Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randhall, R. J (1951) Protein measurement with the folin phenol reagent, *J.biol.Chem*, 193, pp. 265-275.
Oles, J., Dichtl, N. and Niehoff, H. (1997) Full scale experience of two stage thermophilic/mesophilic sludge digestion, *Wat. Sci. Tech .*, Vol. 36, pp. 449-456.
Pagilla,R., Craney,C. and Kido,W. (1997) Causes and effects of foaming in anaerobic sludge digesters, *Wat. Sci. Tech.*, Vol. 36, pp. 463-470.
Tran, F.T. and Tyagi, R. (1990) Mesophilic and thermophilic digestion of municipal sludge in a deep-shaft U-shaped Bioreactor, *Wat. Sci. Tech.*, Vol. 22, pp. 205-215.
Wang, Q.,Chen, J., Kakimoto, K., Ogawa,H. and Kato.Y. (1995) Pretreatment of waste activated sludge results in enhancement of its anerobic digesting efficiency, *Journal of Japan Society on Water Environment*, Vol. 18, pp. 875-882.
Yasui,H., Nakamura, K., Sakuma,S., Iwasaki,M. and Sakai,Y (1996) A full-scale operation of novel activated sludge process without excess sludge production, *Wat. Sci. Tech.*, Vol. 34, pp. 395-404.