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# Microbial growth reduction in sewage sludge by stirred ball mill disintegration and estimation by respirometry

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## Abstract

**BACKGROUND: Excess biomass generation in wastewater treatment plants is an unavoidable byproduct of the degradation process. Treatment and disposal of sewage sludge from wastewater treatment plants accounts for about half or even up to 60% of the total operating cost. The present study focuses on the reduction of excess sludge generation by engineering the microbes through mechanical energy inputs by means of stirred ball milling.**

**RESULTS: The significant achievement of this study is microbial growth reduction of up to 89% at an applied specific energy of 15301 kJ kg$^{-1}$. The degree of disintegration of microbial cells was substantiated by estimating the degree of inactivation ($DD_O$), degree of soluble chemical oxygen demand (COD) release ($DD_{COD}$), particle size and microscopic examination. The effect of disintegration on sludge microbial growth reduction is proved by respirometric studies.**

**CONCLUSIONS: Sludge disintegration employing stirred ball milling is proved to be a worthwhile pretreatment process for reducing the sludge microbial growth in activated sludge treatment processes.**

**Keywords:** respirometry; conversion yield; mechanical disintegration; oxygen uptake rate; stirred ball mill; sludge growth reduction

## LIST OF SYMBOLS

| | |
|---|---|
| $COD$ | soluble chemical oxygen demand [mg L$^{-1}$] |
| $COD_{ut}$ | chemical oxygen demand of untreated sludge [mg L$^{-1}$] |
| $COD_{tr}$ | chemical oxygen demand of treated sludge [mg L$^{-1}$] |
| $COD_{NaOH}$ | chemical oxygen demand release by NaOH hydrolysis method [mg L$^{-1}$] |
| $DD_{COD}$ | degree of disintegration by COD release [%] |
| $DD_O$ | degree of disintegration by inactivation [%] |
| $OUR$ | oxygen uptake rate [mg L$^{-1}$ min$^{-1}$] |
| $OUR_{endo}$ | oxygen uptake rate in endogenous phase [mg L$^{-1}$ min$^{-1}$] |
| $OUR_{tr}$ | oxygen uptake rate of treated sludge [mg L$^{-1}$ min$^{-1}$] |
| $OUR_{ut}$ | oxygen uptake rate of untreated sludge [mg L$^{-1}$ min$^{-1}$] |
| $Y_H$ | heterotrophic conversion Yield [−] |
| $Y_{H(tr)}$ | heterotrophic conversion yield of treated sludge [−] |
| $Y_{H(ut)}$ | heterotrophic conversion yield of untreated sludge [−] |
| $\Delta Y_H$ | sludge growth reduction [%] |
| $TS$ | total solids [g kg$^{-1}$] |
| $oTS$ | organic total solids [g kg$^{-1}$] |
| $O_2$ | oxygen concentration [mg L$^{-1}$] |
| $T$ | time [s] and [min] |
| $E_{spec}$ | specific energy [kJ kg$^{-1}$] |
| $M$ | torque with load [N m] |
| $M_0$ | torque without load [N m] |
| $V$ | volume of the sludge [L] |

## INTRODUCTION

Mechanical wet sludge disintegration is an innovative process for excess sludge reduction. Excess sludge production seems to be an unavoidable drawback of biological wastewater treatment plants. The sludge is produced by the growth of micro-organisms in the

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aeration tank while removing the pollution. Due to the stringent effluent criteria and restrictions on sending excess sludge to landfill, processing and disposal is becoming a more difficult and complex problem. Moreover, the cost of excess sludge treatment and disposal can account for up to 60% of the total operating cost.[1] In addition, there are many new treatment plants in the commissioning stage, and the volume of sewage sludge is expected to increase by at least 50%, leading to 10.1 million tonnes of dry solids per year. In some countries, especially in European countries,[2] sewage sludge will increase by nearly 300%. The expected increase is itself a challenge for waste management and the choices of treatment and disposal methods will have large economic and environmental implications. The landfill of sewage sludge is restricted to prevent health risks to humans and livestock due to potentially toxic elements in the sewage sludge, namely heavy metals, pathogens and organic pollutants.[3] Decline in available land space coupled with increasingly stringent regulations governing the design and operation of new landfills, have caused the cost of siting, building and operating new landfills to rise sharply.[2] An alternative technology, such as incineration, which reduces the sludge to ash is not cost effective. Currently, recycling of sewage sludge mainly by spreading on soil as fertilizer for agriculture or forestry is on the increase, however, the focus on producing clean and non-contaminated food for human consumption will oppose any such idea.[2] Ultimately, it can be concluded that there are limited possibilities for disposal of wastes that can be put into the earth, water or air. In practice none of the methods available is final. In this context, there is an increased interest in the reduction of excess sludge production while retaining the effluent quality criteria. Therefore, efforts have been undertaken to reduce the excess sludge production in the activated sludge process.

In the present work disintegration employing stirred ball milling (SBM) is added as an operational unit in a wastewater treatment plant. The novelty of this work is not only the disintegration of the floc but simultaneously causing damage and rupture to the microbial cells by giving moderate specific energy inputs. Thus, the amount of excess biological sludge produced is reduced without causing deterioration of the treatment plant effluent. A respirometric procedure has been introduced to estimate the heterotrophic conversion yield ($Y_H$) of the sludge. The method analyses the value of the oxygen uptake rate (OUR) versus the amount of substrate. The purpose of this protocol is to assess the potential effect of stress application on cellular maintenance. If maintenance is affected, the energy required for non-growth activities will be increased. Thus, an increased amount of substrate converted to carbon dioxide (associated with oxygen consumed) is expected. By using a respirometric technique in sequenced batch respirometers, $Y_H$ for both treated and untreated sludge is estimated.

## PREVIOUS STUDIES

### Sludge reduction in biological wastewater treatment plant by mechanical disintegration

A new approach was developed to reduce excess sludge by ozonation to achieve complete elimination of sludge production.[4,5] Studies carried out to investigate the maintenance and cryptic growth phenomena of *Pseudomonas fluorescens* showed that viability and sludge production yield decreased with aging of the sludge. Variation of cell content ratio was also observed after discontinuous thermal treatment.[6]

The effect of maintenance energy requirements on biomass production revealed possible methods to reduce the excess biomass during the treatment process.[7] Comparisons of different strategies for reducing excess sludge production and their merits and demerits have been clearly presented.[8] Another new approach, that of increasing the oxygen concentration in activated sludge flocs, led to minimization of excess sludge production.[9] Reduction in excess sewage sludge was found to be possible by integrating the mechanical disintegration of sludge into the activated sludge process.[10] Studies on mechanical disintegration and ozonation of excess sludge showed accelerated anaerobic digestion.[11] Besides mechanical disintegration and ozonation, other methods, e.g. oxidation, thermal, chemical and thermochemical pretreatment methods are also used to improve sludge digestibility.[12–14]

### Stirred ball mill method

Experimental investigations using SBM disintegration have been carried out by several researchers.[10,11,15] Most studies are focused on rupture of the whole cells. A review work[16] clearly recommends that the high pressure homogenizer and bead mill are the most suitable for large-scale microbial cell disruption for the production of intracellular enzymes and organelles. The method was employed to prove that the yield of intracellular enzyme activity could be maximized by complete cell disruption[17,18] by means of a ball mill and a cutter mill. Of the two methods employed, it was found that ball milling yielded better disintegration results, perhaps due to the sequential process: breaking up microbial cell walls, releasing intracellular carbon compounds and nutrients for further anaerobic digestion. Mueller[19] investigated various methods of mechanical disintegration and showed that all methods are able to break up the floc but only some of them provide enough energy for disruption of the micro-organisms.

### Respirometric studies

Literature sources describe the use of respirometric methods for various applications. To study the effect of the anaerobic biological process, on the removal of phosphorus a respirometric method was used to determine the readily biodegradable chemical oxygen demand (COD) concentration.[20] Similarly, a respirometric method was proposed to estimate slowly biodegradable COD and the active heterotrophic biomass present in wastewater.[21] A respirometry technique was also employed to assess the storage yield for different substrates, to predict and control the activated sludge process, as well as for the measurement of substrate concentration in wastewater.[22] Further, this technique has also been used for biological characterization as it assesses the biological behavior of the pollution and provides a rapid tool for wastewater and activated sludge characterization.[23] To promote the conversion of organic pollutants to respiration products with the concomitant increase in aeration requirements,[24] a respirometric procedure was introduced to estimate the heterotrophic conversion yield ($Y_H$) of the sludge.

## MATERIAL AND METHODS

The sewage sludge used in this study was collected from the outlet of the aeration tank of the municipal wastewater treatment plant in Braunschweig, Germany. Sludge disintegrability studies were conducted at a laboratory scale of 3 L with sludge that contains total solids (TS) of 3.5 g kg$^{-1}$. Disintegrability experiments were carried out in batch mode using a SBM (Model LME 4, Netzsch, Feinmahl Technik GmbH, Germany) at 1400 rpm for 20 min. Intermediate samples were taken for COD and $DD_O$ analysis. A mean bead size of 770 µm diameter was used in the ball mill. The grinding chamber of the ball mill was provided with a cooling jacket through which coolant was passed to maintain a temperature of 14 °C (±1 °C). This is done to take care of the rise in temperature as a result of grinding, which may affect cell rupture to some extent. The mill was operated at 1100 rpm with treatment time up to 20 min. Intermediate samples (150 mL) were collected at specific intervals and each time the same volume of sludge was replaced by fresh sludge. After disintegration, the outlet temperature of the sludge was found to be less than 20 °C.

Floc rupture was measured by analyzing the particle size for the treated and untreated sludge using a laser diffraction analyzer (Helos 12 KA, Sympatec, Germany).

Microscopic examination of treated and untreated sludge samples was carried out with a phase contrast microscope (Olympus, Japan) at a magnification of 1000×.

The degree of disintegration can be determined by two biochemical parameters; degree of inactivation ($DD_O$) can be determined by measuring the OUR of the treated ($OUR_{tr}$) and the untreated sludge ($OUR_{ut}$). This can be calculated as follows:

$$DD_o[\%] = \left[1 - \frac{OUR_{tr}}{OUR_{ut}}\right].100 \qquad (1)$$

The degree of soluble COD release ($DD_{COD}$) can be determined by normal COD analysis. Before COD analysis the sludge samples are centrifuged at 10 000 g and the supernatant is filtered using a cellulose nitrate membrane with pore size 0.45 µm by compression. The filtered liquid is subjected to COD analysis as per the standard procedure,[25] The degree of disintegration by soluble COD release ($DD_{COD}$) can be determined by the following equation:

$$DD_{COD}[\%] = \left[\frac{COD_{tr} - COD_{ut}}{COD_{NaoH} - COD_{ut}}\right].100 \qquad (2)$$

### Respirometer

The experimental setup as shown in Fig. 1 for respirometry used in these studies is similar to the setup reported elsewhere[21,26] and the following procedure was adopted. The experiments were performed in a 2.5 L capacity cylindrical aerated reactor (100 mm diameter, 355 mm height) with

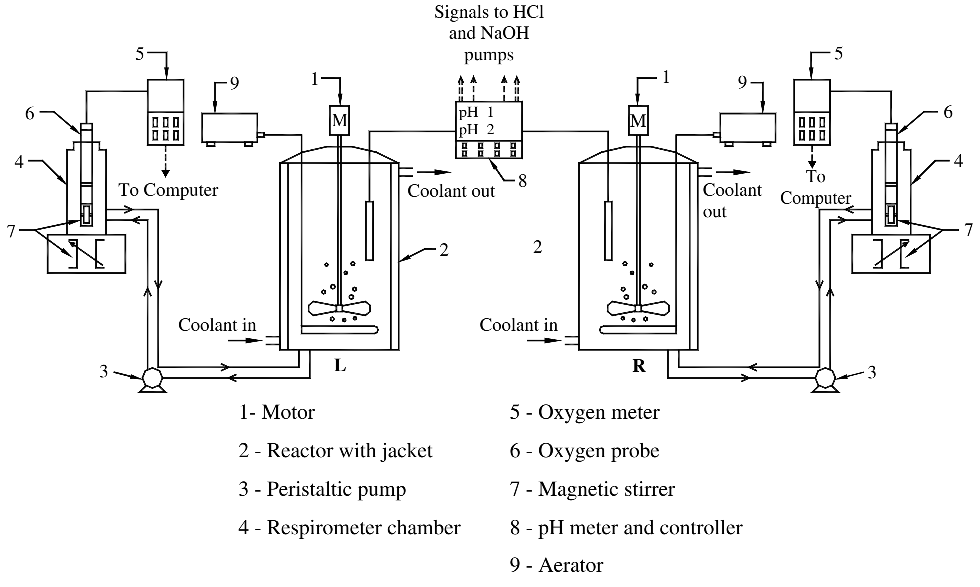

**Figure 1.** Experimental setup for respiration measurements.

working volume 1.8 L. The reactors were stirred at 300 rpm by means of a motor (IKA Eurostar 130 W) equipped with 4-pitched blades, 60 mm diameter. The reactor was double jacketed and the temperature of the sludge was maintained at 20 °C (±2 °C). pH controllers were installed in both reactors and pH was kept between 7 and 7.5 by adding acid or base as needed, by means of a peristaltic pump. The sludge from the reactor was pumped into a respirometric chamber (cylindrical, capacity of 0.08 L, stirred and air tight vessel) for OUR measurement using a peristaltic pump. The temperature and dissolved oxygen concentrations were measured in the respirometric chamber by means of a dual sensor (model cell-ox 325, WTW, Weilheim, Germany) attached to an oxygen meter (model oxi 340i, WTW). The accuracy of the dissolved oxygen measurement was ±0.5% of the oxygen concentration value. The oxygen meters were connected to a computer by means of RS 232 cable to continuously monitor and recording the data. Sufficient aeration was given and the oxygen concentration maintained $>5\,\mathrm{mg\,L^{-1}}$ so that the micro-organisms would not suffer lack of oxygen while the degradation process took place.[9,21] During the measurement of OUR the pump circulation was stopped to ensure that no mass transfer occurred. OUR can be computed by measuring the decrease in dissolved oxygen over time, as follows:[23]

$$OUR = -\frac{d[o_2]}{dt} \tag{3}$$

The vessel was automatically re-aerated for each new OUR measurement.

## Batch respirometric measurements

The sludge, containing TS of $3.5-4.5\,\mathrm{g\,kg^{-1}}$ was taken from the outlet of the aeration tank for respiration measurements. Prior to measuring the initial OUR profile, $20\,\mathrm{mg\ L^{-1}}$ of allyl thio urea was added to the sludge to inhibit nitrification.[21] Then the sludge sample was aerated for 12 h to degrade all degradable substrates within the sludge. At this stage to control foaming, $0.7\,\mathrm{mL\ L^{-1}}$ antifoaming agent (AGITAN 301, Munzing Chemicals, Germany) was added to the reactor and the respiration rate measured. This respiration rate is termed $OUR_{endogenous}$. (To identify suitable antifoam, screening experiments were carried out along with respiration measurements in the presence of several antifoams to make sure that there was no inhibition in respiration due to the antifoaming agent. The antifoaming agent employed in this study is biodegradable). Then the sludge was removed from the respiration reactor and treated with SBM at 1500 rpm. Subsequent to SBM treatment, the samples for $DD_O$, COD release analysis were collected.

A freshly prepared synthetic wastewater, according to the modified specifications laid out by Organization for Economical Co-operation and Development (Ginestet P, personal communication, OECD modified, INSA, Toulouse, France) was added as a substrate as a concentrated solution of 25 mL in both reactors. (The composition of the synthetic wastewater comprised sodium acetate 0.79 g, peptone 0.79 g, meat extract 0.5 g, $(NH_4)_2SO_4$ 0.9 g, NaCl 0.09 g, $CaCl_2$ 0.05 g, $MgSO_4\,7H_2O$ 0.03 g and $K_2HPO_4$ 0.35 g. These items were mixed homogeneously in 65 mL of de-ionized water.) The substrate COD concentration was found to vary between 410 and $445\,\mathrm{mg\ L^{-1}}$ from batch to batch. After the addition of substrate, OUR was measured for the degradation process. As the degradation process proceeds, OUR first increases and later decreases to the initial value. The time, at which the substrate is added is referred as to $t_1$, while the time at the end of the degradation process (endogenous phase) is referred to as $t_2$. The COD of the sludge liquid was analyzed before the addition of substrate and also after reaching the endogenous phase (indicated by constant OUR). Thus from the difference in COD values, the amount of degraded COD can be calculated (ie. $COD_{initial} + COD_{substrate} - COD_{end}$). In addition, sludge TS and organic total solids (oTS) before $t_1$ and $t_2$ (i.e. sludge samples collected after 20 h of aeration with OUR measurements) were weighed with a precision of 0.0001 g for both treated and untreated sludge to characterize growth of micro-organisms based on *Standard Methods for the Examination of Water and Wastewater*, 20th edn (AWWA), 1999.

## Specific energy (*E<sub>spec</sub>*)

The definition of specific energy is the energy input in relation to the treated solid mass, which provides information about the necessary energy input for the disintegration process to achieve a certain degree of disintegration. This can be calculated by the equation

$$E_{spec} = \frac{(M-M_0)2\pi n}{V_* TS} \tag{4}$$

where $M$ and $M_0$ are the torque recorded when the ball mill is operated with and without the sludge load and $n$ is the absolute revolutions of the agitator. The treated mass can be found by measuring the volume of the sample $V$ and the total solids content $TS$.

## Conversion yield ($Y_H$)

Conversion yield ($Y_H$) can be used to assess the potential effect of stress application on cellular maintenance. If the maintenance is affected, the energy required for no growth reactions should be increased and at the same time $Y_H$ has to decrease. The conversion of a known amount of biodegradable substrate into carbon dioxide allows an estimation of $Y_H$. Using the OUR profile the conversion yield can be calculated by employing the following equation for both treated and untreated sludge.

$$Y_H = 1 - \frac{\int_{t_1}^{t_2} (OUR_{total} - OUR_{endo})dt}{COD_{removed}} \tag{5}$$

### Sludge growth reduction

The sludge growth reduction can be calculated using the following equation:

$$\Delta Y_H[\%] = \left[1 - \frac{Y_{H(tr)}}{Y_{H(ut)}}\right].100 \qquad (6)$$

where the subscripts *tr* and *ut* denote treated and untreated sludge.

## RESULTS AND DISCUSSIONS

### Influence of specific energy input on particle size distribution

The disintegration of sludge can be described by particle size analysis. It can be seen from Fig. 2 that particle size reduces as the energy input increases. It is observed that the major size reduction takes place at an energy input of 5035 kJ $kg^{-1}$ and further increase in energy input to 8801 and 35 204 kJ $kg^{-1}$ results in only minor further size reduction.

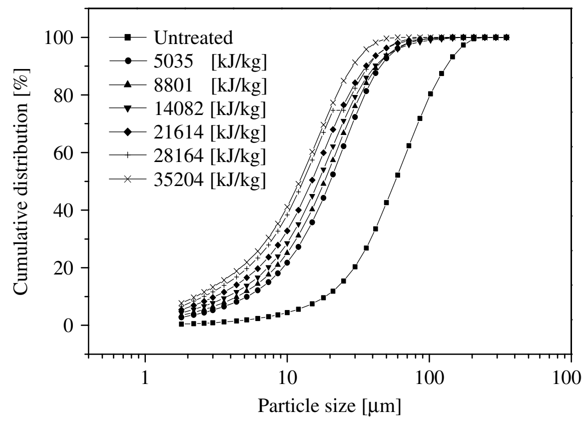

**Figure 2.** Reduction of particle size achieved with stirred ball mill as a function of specific energy input.

### Microscopic examination of untreated and treated sludge

Microscopic examinations of the untreated sludge and treated sludge floc with different specific energy inputs are shown in Fig. 3, slides A–F. Slide A is a typical micrograph of the untreated sludge. Slide B is a micrograph of the sludge treated at 5035 kJ $kg^{-1}$. A comparison of this micrograph with slide A shows a major size reduction at the specific energy 5035 kJ $kg^{-1}$. Slides C–F show the micrographs of sludge treated with increasing energy inputs. In these

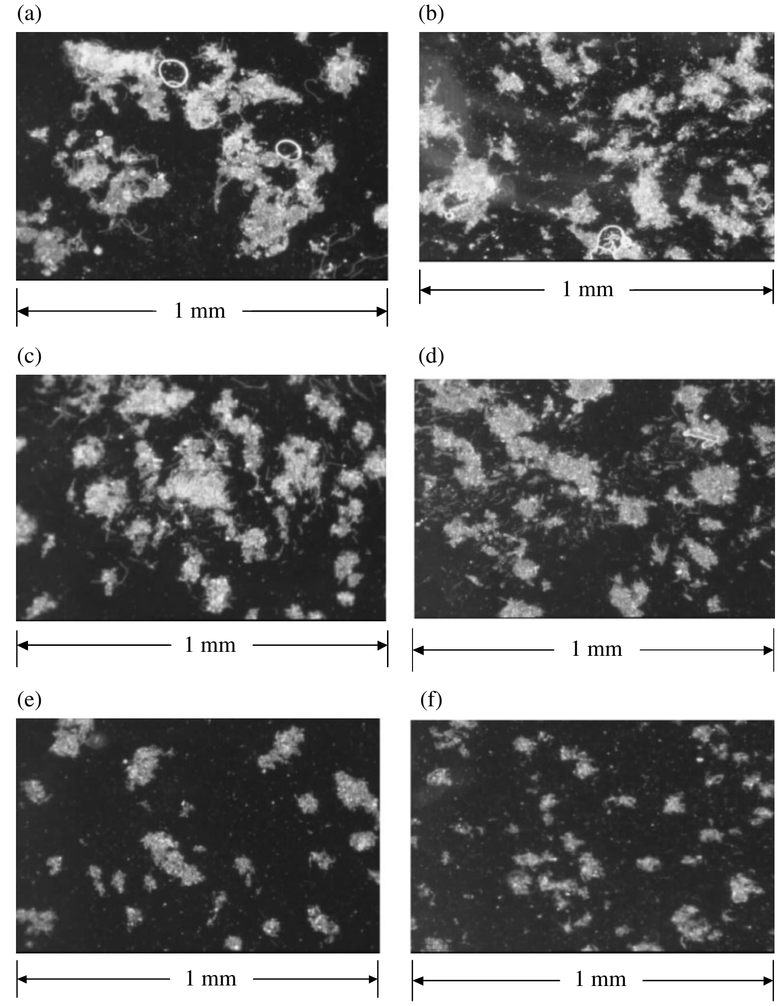

**Figure 3.** Slide A: original sludge floc; Slide B: sludge treated at 5035 kJ $kg^{-1}$; Slide C: sludge treated at 8801 kJ $kg^{-1}$; Slide D: sludge treated at 21 614 kJ $kg^{-1}$; Slide E: sludge treated at 28 164 kJ $kg^{-1}$; Slide F: sludge treated at 35 204 kJ $kg^{-1}$.

micrographs the porous structure with filaments is indicative of filamentous bacteria. It can be seen that on increasing the energy, the floc structure loosens as a result of disintegration. At 8801 kJ kg$^{-1}$ energy input and beyond, the filamentous material present in the sludge floc fragments into smaller lengths, resulting in size reduction. At 28 164 and 35 204 kJ kg$^{-1}$ the filamentous material is destroyed and maximum size reduction of the floc is observed. Thus, microscopic examination of treated and untreated sludge clearly supports the idea of size reduction with increasing specific energy. However, the major size reduction takes place at low applied energy of 5035 kJ kg$^{-1}$, further increase in the specific energy input results in only to minor reductions in size. The microscopic investigation supports the particle size studies reported above.

## Degree of disintegration and respirometric studies

*Degree of disintegration by stirred ball mill with increasing specific energy inputs*

Figure 4 shows the result of the disintegrability studies (DS) at various specific energy inputs. A retention time of 3 min for the sludge in the ball mill corresponds to a specific energy of 5000 kJ kg$^{-1}$ using Eqn (4). At this specific energy input it is seen that the microorganisms are subjected to less strain (pressure and shear forces) and hence only 30% inactivation is measured. As the residence time for the sludge in the ball mill is increased, the obvious increase in energy input results in 37–80% inactivation, as shown in Fig. 4. The corresponding COD release observed is 10% at 5000 kJ kg$^{-1}$ and reaches a maximum of 23.8% at the maximum energy input. The COD release gradually increases as the energy input increases, as for inactivation. Thus, on increasing the specific energy, a consequent increase in inactivation and COD release occurs without rupturing all the microbial cells in the sludge.

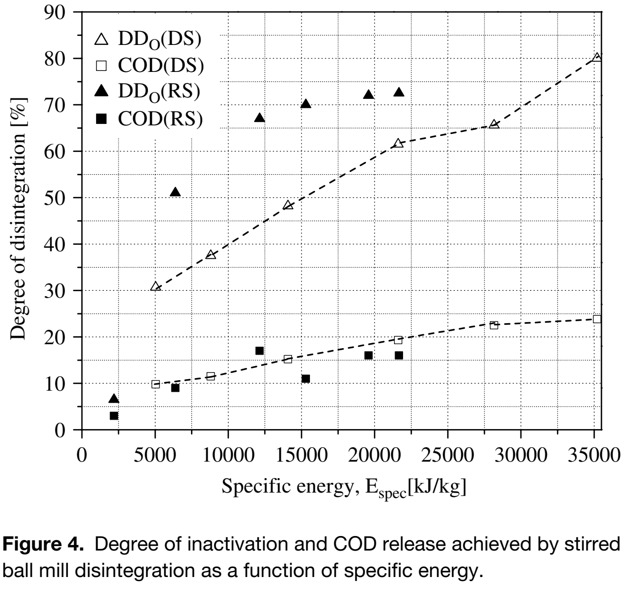

**Figure 4.** Degree of inactivation and COD release achieved by stirred ball mill disintegration as a function of specific energy.

The objective of carrying out respirometric studies is to asses the oxidative efficiency of the sludge treated by SBM. Figure 4 shows the results for different respirometric studies (RS) for SBM-treated sludge at various specific energies. It can be seen that the respirometric trend results follow the observations of the inactivation and COD studies. A lower inactivation of 6.5% is attained at a lower energy of 2184 kJ kg$^{-1}$, while 72.5% inactivation is seen at 19 703 kJ kg$^{-1}$. Thus, an increasing trend in microbial inactivation is recorded with increased energy inputs. COD release also follows the same trend; the maximum COD release at maximum energy input 19 703 kJ kg$^{-1}$ is 16%. This observation supports the fact that SBM within the specific energy limits (2184–19 702 kJ kg$^{-1}$) influences the reduction in particle size, cell damage and cell ruptures. It can be concluded that SBMl appears to be an efficient method for disintegrating the floc and for cell disruption in a wastewater degradation process.

Incorporating the proposed SBM in the wastewater treatment was explored. Figure 5 depicts the inclusion of SBM as an operational unit in the WWTP for sludge disintegration. The sludge that will be accumulated in the clarifier can be returned through SBM to the aerobic basin. This operation can be routinely carried out to minimize the excess sludge.

*Sludge microbial growth reduction and estimation of heterotrophic conversion yield*

A comparison of OUR profiles recorded for the untreated sludge and treated sludge at increasing specific energy inputs (2184, 6378, 12 148, 15 301, 19 702 kJ kg$^{-1}$) are shown in Fig. 6(A)–(D). In these figures a clear difference in OUR profiles as a result of SBM treatment can be seen. This is confirmed by estimating the area of $OUR_{endogenous}$ from $OUR_{total}$. It is found that the area of $OUR_{exogenous}$ for the untreated sludge is smaller than that for the treated sludge. The conversion yield is calculated employing Eqn (5) and the data is summarized in Table 1. The COD data of untreated and treated sludge before and after the respirometric study is also reported in Table 1. An examination of Table 1 reveals that on increasing specific energy the yield coefficient $Y_H$ for the treated sludge decreases from 0.33 to 0.07

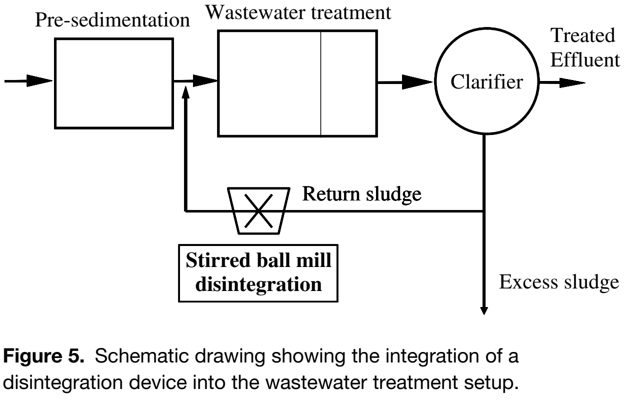

**Figure 5.** Schematic drawing showing the integration of a disintegration device into the wastewater treatment setup.

**Table 1.** Parameters of the sludge treated by SBM at 1500 rpm (specific energy, degree of inactivation, degree of COD release, initial and final CODs of untreated and treated sludge and COD removed) during respirometric measurements and calculated conversion yields

| Specific energy [kJ kg$^{-1}$] | Sludge category | $COD_{initial}$ [mg L$^{-1}$] | $DD_O$ [%] | $DD_{COD}$ [%] | $COD_{final}$ [mg L$^{-1}$] | COD removed [mg L$^{-1}$] | $Y_H$ [–] | Growth reduction $\Delta Y_H$ [%] |
|---|---|---|---|---|---|---|---|---|
| 2184 | Untreated | 70 | – | – | 68 | 426 | 0.44 | 25 |
| | Treated | 160 | 6.5 | 3 | 93 | 491 | 0.33 | |
| 6378 | Untreated | 72 | – | – | 79 | 408 | 0.43 | 37 |
| | Treated | 330 | 51 | 9 | 123 | 622 | 0.27 | |
| 12 148 | Untreated | 77 | – | – | 77 | 445 | 0.39 | 42 |
| | Treated | 515 | 69 | 17 | 186 | 744 | 0.22 | |
| 15 301 | Untreated | 84 | – | – | 73 | 435 | 0.61 | 89 |
| | Treated | 442 | 70 | 11 | 123 | 743 | 0.07 | |
| 19 702 | Untreated | 86 | – | – | 75 | 421 | 0.50 | 78 |
| | Treated | 552 | 72 | 16 | 176 | 786 | 0.11 | |

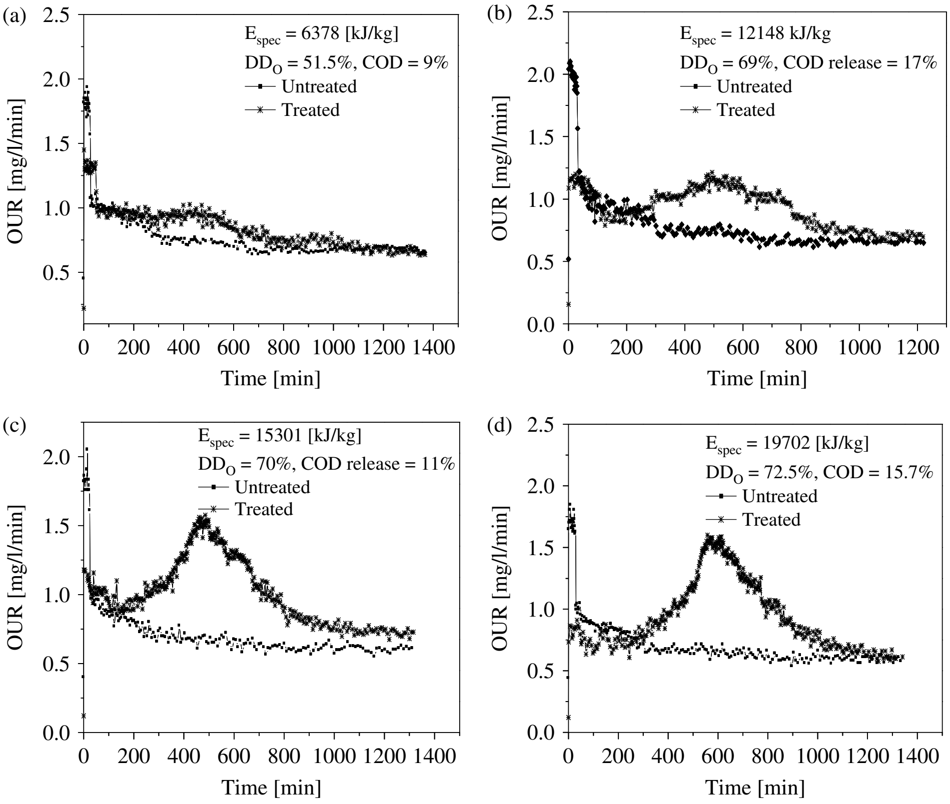

**Figure 6.** Comparison of OUR profiles of treated and untreated sludge. Energy input (A) 6378 kJ kg$^{-1}$; (B) 12 148 kJ kg$^{-1}$; (C) 15 301 kJ kg$^{-1}$; (D) 19 702 kJ kg$^{-1}$.

(i.e. for specific energy 2184–15 301 kJ kg$^{-1}$). When the energy input is further increased to 19 702 kJ kg$^{-1}$ there is a slight increase in $Y_H$ to 0.10. This shows that an optimal energy of 15 301 kJ kg$^{-1}$ is sufficient to give low $Y_H$. This trend confirms that there is considerable growth reduction with increasing specific energy up to a level of 15 301 kJ kg$^{-1}$. At this stage it is hypothesized that the reduction in sludge growth is primarily due to maintenance energy requirements of the microbial cells (cell repair) caused by SBM disintegration. During SBM treatment, floc rupture and cell damage trigger and enhance the aerobic oxidative capacity. The enhancement in aerobic oxidative capacity is mainly due to an increase in maintenance energy requirements, as a result of which, substrate consumption occurs competitively for energy maintenance, rather than solely for reproduction.

In Table 2 the biomass concentration of untreated and treated sludge before and after respirometric studies is detailed. One can clearly see from the data that after 22 h of respirometry there is no appreciable growth (i.e. increase in biomass). This is because, on treatment, the micro-organisms are stressed and do not involve themselves in cell synthesis for growth but in repairing the damage cause by the treatment, i.e. maintaining cell integrity. Similar observations are well reported in the literature.[27] When viewed on the basis of biomass, there is insignificant sludge reduction. However, the important point to be noted is that the mechanical treatment process facilitated the growth reduction by engineered microbes.

Figure 7 shows the sludge microbial growth reduction (calculated using Eqn (6)) as a function of specific energy. A reducing trend of sludge growth is obtained when the energy input is increased. This confirms that the sludge growth reduction mechanism under SBM treatment is mainly due to the increase in cellular maintenance energy requirements. Another reason for the decrease in sludge growth with increase in specific energy is the decrease in particle size. Decreased particle size of the sludge floc can facilitate micro-organism participation in the metabolic activity. A growth reduction of 89% was achieved (Table 1) at a specific energy of 15 301 kJ $kg^{-1}$. Thus, it has been proved beyond doubt that incorporation of SBM pretreatment in WWTP reduces sludge growth by up to 89%.

SBM treatment disintegrates the floc, damages the cells and causes rupture of the cell wall of many cells, leading to an increase in maintenance energy demand. This forces the strained cells to consume the substrate, competiting for maintenance metabolism. In addition, floc rupture releases micro-organisms and facilitates the substrate diffusion process. Although several researchers[12,15] have used different mechanical treatment processes in the past and demonstrated sludge reduction in the sludge treatment process (i.e. before disposal), here, for the first time, it has been proved that by disintegrating the aerobic sludge using moderate energy inputs during wastewater degradation so that floc rupture, cell damage and cell rupture occurs simultaneously, sludge microbial growth is reduced. This is a specific manipulation for increased cellular maintenance of micro-organisms, which in turn increases the oxidative capacity of the micro-organisms.

**Table 2.** Biomass concentration of untreated and treated sludge before and after the respirometric studies

| Specific energy [kJ $kg^{-1}$] | Sludge category | Initial biomass concentration [g $kg^{-1}$] | Biomass concentration after 22 h [g $kg^{-1}$] | Sludge reduction on biomass basis [%] |
|---|---|---|---|---|
| 2184 | Untreated | 3.1311 | 3.2020 | 2 |
| | Treated | | 3.1370 | |
| 6378 | Untreated | 3.1678 | 3.2305 | 4.7 |
| | Treated | | 3.0790 | |
| 12 148 | Untreated | 3.0757 | 3.2570 | 5.1 |
| | Treated | | 3.0896 | |
| 15 301 | Untreated | 3.3813 | 3.4618 | 6.2 |
| | Treated | | 3.2485 | |
| 19 702 | Untreated | 3.1325 | 3.2268 | 7.3 |
| | Treated | | 2.9892 | |

## Influence of *DD*$_O$ and soluble COD release on microbial sludge growth

Sludge microbial inactivation is one of the parameters used in sludge growth reduction. This is accomplished by releasing the micro-organisms from the floc, stressing the organisms either by damage to, or total rupture of, the cell. From Fig. 8, the reduction in sludge microbial growth can be seen with increasing degree of inactivation. At 6.5% inactivation disintegration is not sufficient and reduces growth by only 25%. In the range 51.5–70% inactivation, a growth reduction of 37–89% was achieved. It can be seen from Fig. 8 that any further inactivation decreases growth reduction; e.g. at 72% inactivation, the growth reduction is reduced to 78%. Therefore it can be concluded that excessive inactivation will not achieve maximum growth reduction.

For microbial growth reduction, floc rupture and inactivation alone are not sufficient; release of degradable COD (i.e. cell rupture) is also important. In Fig. 9 growth reduction with respect to COD release is shown. It can be seen that 25% reduction in sludge growth occurs for 3% COD release. Further, release of 9–11% COD enhances growth reduction to a maximum of 89%. This shows that at a moderate energy input of 15 301 kJ $kg^{-1}$ both the COD release

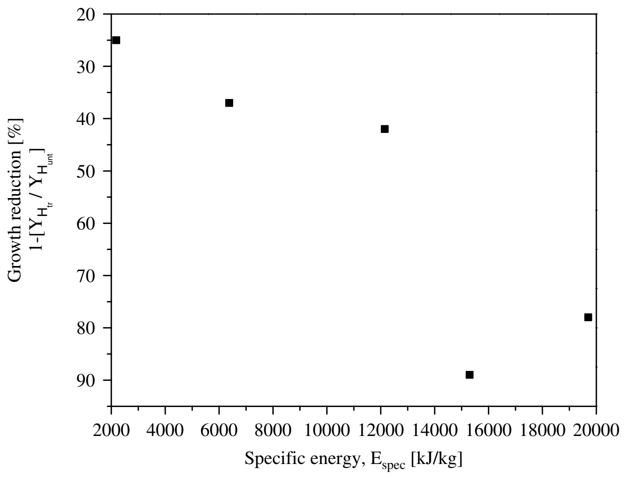

**Figure 7.** Influence of specific energy on sludge microbial growth reduction.

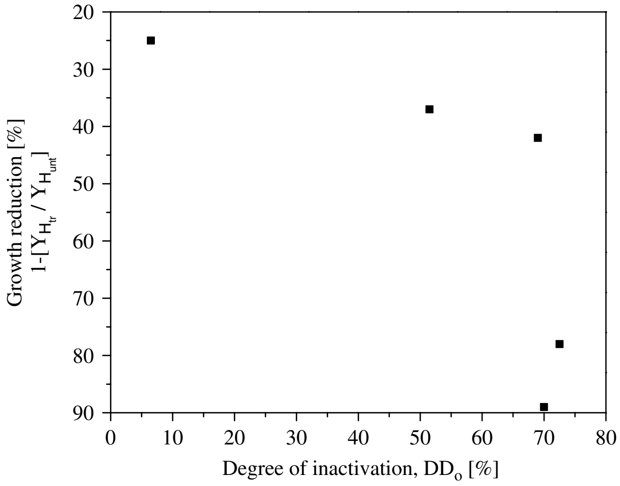

**Figure 8.** Influence of degree of inactivation on sludge microbial growth reduction.

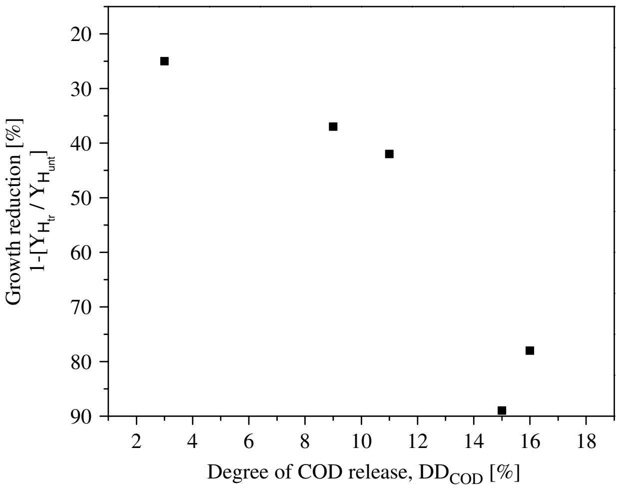

**Figure 9.** Influence of degree of soluble COD release on sludge microbial growth reduction.

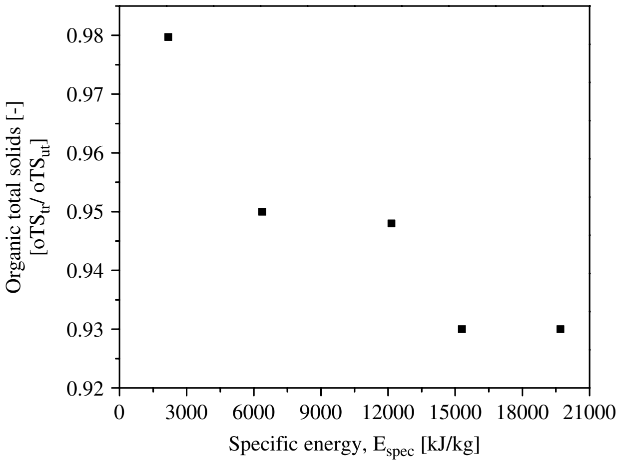

**Figure 10.** Influence of specific energy on organic total solids.

and the ground material facilitates the degradation process. Further increase in energy input to 19 702 kJ $kg^{-1}$, although increasing COD release, decreases sludge growth reduction. This may be because there are many dead cells and greater release of slowly degradable material, including cell debris, at higher specific energy inputs.

### Reduction of organic total solids

The respirometric studies of untreated and treated sludge samples were analyzed for oTS employing a standard gravimetric method (Ginestet P, personal communication). In Fig. 10 the ratio of the organic total solids is plotted against specific energy input. It is clear from the figure that there is a mass reduction and changes of cell viabilities as a result of SBM disintegration, indicated by the decrease in oTS concentration with increase in specific energy inputs.

## CONCLUSIONS

Sludge disintegration employing SBM is proved to be a worthwhile pretreatment process for reducing sludge microbial growth in activated sludge treatment processes. The soluble COD increase represents sludge disintegration, which releases organic matter from the microbial floc into the water. Microbial inactivation is determined from the decrease in biological activity (OUR). A decrease in oTS shows the mass reduction due to SBM disintegration. The study has shown that at a specific energy input of 15 301 kJ $kg^{-1}$ as much as 89% reduction in growth can be achieved. In this study the sludge growth reduction has been proved by means of sequenced batch respirometric studies, degree of disintegration, particle size and microscopic investigations. The mechanistic aspect of sludge microbial growth reduction by means of applied mechanical energy is also discussed in detail. The incorporation of ball milling in wastewater treatment plants is proposed.

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