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1 **Comparison between disintegrated and fermented sewage sludge for production**
2 **of a carbon source suitable for biological nutrient removal**

3
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11

12 **Abstract**

13 There is a need to investigate processes that enable sludge re-use whilst enhancing sewage
14 treatment efficiency. Mechanically disintegrated thickened surplus activated sludge (SAS) and
15 fermented primary sludge were compared for their capacity to produce a carbon source suitable for
16 BNR by completing nutrient removal predictive tests. Mechanically disintegration of SAS using a
17 deflaker enhanced volatile fatty acids (VFAs) content from 92 to 374 mg l⁻¹ (4.1 fold increase). In
18 comparison, primary sludge fermentation increased the VFAs content from 3.5 g l⁻¹ to a final
19 concentration of 8.7 g l⁻¹ (2.5 fold increase). The carbon source obtained from disintegration and
20 fermentation treatments improved phosphate (PO₄-P) release and denitrification by up to 0.04 mg
21 NO₃-N g⁻¹ VSS min⁻¹ and 0.031 mg PO₄-P g⁻¹ VSS min⁻¹, respectively, in comparison to acetate
22 (0.023 mg NO₃-N g⁻¹ VSS min⁻¹ and 0.010 mg PO₄-P g⁻¹ VSS min⁻¹). Overall, both types of sludge
23 were suitable for BNR but disintegrated SAS displayed lower carbon to nutrient ratios of 8 for
24 SCOD:PO₄-P and 9 for SCOD:NO₃-N. On the other hand, SAS increased the concentration of PO₄-

1 P in the settled sewage by a further $0.97 \text{ g PO}_4\text{-P kg}^{-1}$ SCOD indicating its potential negative impact
2 towards nutrient recycling in the BNR process.

3 **Keywords:** denitrification; mechanical disintegration; phosphorus release; primary sludge; surplus
4 activated sludge

5

6 **1. Introduction**

7 Removal of phosphorus (P) and nitrogen (N) from wastewater effluents is important to ensure
8 environmental protection of surface waters. High concentrations of nutrients in rivers have been
9 related to eutrophication [1]. Biological nutrient removal (BNR) processes are widely used to
10 remove nutrients from wastewater, however for the phosphate accumulating organisms (PAO) and
11 heterotrophic denitrifiers to be able uptake P and reduce nitrate (NO_3^-) respectively, the wastewater
12 must have sufficient carbon to favour their metabolism [2]. It has been reported that 6-10 mg of
13 volatile fatty acids (VFAs) or 20 mg chemical oxygen demand (COD) as acetic acid are required to
14 remove 1 mg of phosphorus [2] and that 3-4 mg COD as acetic acid per 1 mg of total nitrogen are
15 required for denitrification [3]. More recently, a study in a sequencing batch biofilm reactor
16 demonstrated that 7 mg l^{-1} of acetate are required to remove 1 mg P l^{-1} [4]. To achieve the essential
17 carbon to nutrient ratio, the wastewater can be supplemented with an industrial effluent rich in
18 soluble carbon or chemicals such as ethanol, methanol or acetate to the wastewater. However, the
19 dependence of BNR over the existence of local industries or the costs associated with transport and
20 chemical storage facilities can be significant, decreasing the attractiveness of these external carbon
21 sources. Alternatively, a carbon source rich in VFAs, which are the most suitable carbon source for
22 BNR [5], can be produced at the sewage treatment works (STWs) by disintegration or fermentation
23 of sewage sludge. This approach can stimulate sludge re-use as an alternative to anaerobic digestion
24 and potentially improve BNR efficiency [6].

25

1 Nevertheless, the question remains as to what sludge source to use and the appropriate mechanism
2 to apply for VFAs production. Fermentation of primary sludge can promote VFAs production [7, 8]
3 and this method is currently used in countries such as South Africa and the USA for internal carbon
4 source production for BNR [9]. The sludge retention time in the fermenter is critical to achieve high
5 VFAs production in order to enhance hydrolysis and acidogenesis without methane gas formation.
6 Hence, the sludge retention time must be lower than 6 to 10 days depending on the temperature
7 20°C to 10°C, respectively [10, 11]. Fermentation of secondary sludge has been described to be
8 complex due to the composition of the sludge, mainly microbial cells and flocs, which are difficult
9 to breakdown under anaerobic conditions [12-14]. Furthermore, VFAs-COD equivalent production
10 from SAS fermentation has been reported to be low with 21 mg g⁻¹ volatile suspended solids (VSS)
11 compared with primary sludge fermentation 226 mg g⁻¹ VSS [15]. Alternatively the production of
12 an internal carbon source from secondary sludge can be achieved through mechanical
13 disintegration. This has been demonstrated to increase the soluble chemical oxygen demand
14 (SCOD) and VFAs content by disrupting microbial flocs and cells using pressure and friction forces
15 [16, 17]. However, there is little information about the application of the disintegrated sludge to
16 BNR in order to improve P and N removal [18] and how it compares with primary sludge
17 fermentation.

18
19 The aim of this study was to compare fermented primary sludge and disintegrated SAS, both
20 centrifuged supernatant and disintegrated sludge, for producing a carbon source suitable for BNR.
21 The two methods were compared in terms of VFAs production rate, type of VFAs released and
22 SCOD release rates. Furthermore, the nutrient removal potential of the internal carbon sources was
23 determined by completing nutrient removal predictive tests and comparing removal rates with
24 acetate – an external carbon source. The implications of each of the technologies to the overall
25 treatment process were also assessed in relation to the possible internal recycling of nutrients within
26 the STW.

1 **2. Materials and methods**

2 *2.1 Mechanical disintegration of surplus activated sludge*

3 Five litres of thickened surplus activated sludge (SAS) was collected from a 1 m³ BNR pilot-plant
4 and mechanically disintegrated using a 10" Pilao DTD Spider Deflaker with a 30 kW motor fitted
5 with 230 mm discs with 3 active cell layers according to Kampas et al. [16]. The disintegration
6 process was conducted as a batch. A portion of the disintegrated sludge was centrifuged for 20 min
7 at 8804×g (Hettich Zentrifugen, Tuttlingen, Germany). The centrifuged supernatant and
8 disintegrated sludge were used within hours after disintegration for nutrient removal predictive tests
9 and analysed for VFAs, SCOD, nitrate (NO₃-N), ammonium (NH₄⁺) and PO₄-P contents.

10

11 *2.2 Fermentation of primary sludge*

12 Primary sludge was collected from five full-scale STWs (Yorkshire, UK) with population
13 equivalents (PE) of 499,065, 386,123, 373,985, 373,985, 573,394 and 18,914 for Sites 1, 2, 3, 4 and
14 5, respectively. Four and half litres of primary sludge were fermented in 5 l vessels (quickfit flask
15 100 mm flange bore, height 290 mm QRE-130-B, Fisher, UK) for 4 days at room temperature (20-
16 23°C). The sludge was mechanically mixed at a constant speed of 0.7×g by overhead motor stirrers
17 (Heidolph RZR 2020 and 2102, Schwabach, Germany) with anaerobic conditions promoted by
18 sealing the fermentation vessels. Every 12h, 60 ml of sludge was sampled for VFAs analyses and
19 every 24h for total suspended solids (TSS), SCOD, pH, NH₄⁺ and PO₄-P analyses. All
20 fermentations were completed in duplicate. At the end of 80h, the fermentation product obtained
21 from the sludge collected at Site 1 was centrifuged for 20 min at 8804×g (Hettich Zentrifugen,
22 Tuttlingen, Germany) and the supernatant stored frozen at -20°C for nutrient removal predictive
23 tests. This fermentation product was selected because it displayed high VFAs production from an
24 initial concentration 2.2 g l⁻¹ to a final 6.3 g l⁻¹ (2.8 fold increase) and presented typical VFAs
25 contents of fermented primary sludge with 44% acetic acid and 35% propionic acid [19].

1 2.3 Nutrient removal predictive tests

2 Phosphorus release and denitrification testes were conducted in 2.5 l plexi-glass vessels at 25°C
3 according to method described by Kampas et al. [20]. A mixture of 1 l wastewater and 1 l returned
4 activated sludge (RAS), collected from a full-scale BNR plant in Derbyshire, UK was placed in
5 each of the five vessels used for the nutrient removal predictive tests. Nitrogen gas was
6 continuously supplied to the headspace of the vessels to ensure anaerobic conditions. The vessels
7 were mixed with magnetic stirrers at 20.2×g and the pH and dissolved oxygen continuously
8 monitored with stable values at 7.2-7.5 and $< 0.15 \text{ mg l}^{-1}$, respectively. Vessel 1 was used as a
9 control without a carbon source addition, vessel 2 was spiked with a solution of sodium acetate and
10 vessels 3, 4 and 5 were spiked with liquor obtained from the fermented primary sludge,
11 mechanically disintegrated SAS and supernatant from the SAS disintegration, respectively. This
12 experimental configuration was used for a total of 4 different tests for phosphorus release and
13 denitrification when the amount of carbon added to each vessel was matched in SCOD (50 mg l^{-1})
14 and total VFAs (3.5 mg l^{-1}) contents (Table 1).

15
16 For the denitrification tests, $20 \text{ mg KNO}_3 \text{ l}^{-1}$ was added to each vessel at the beginning of the
17 experiment to ensure sufficient nitrate in the wastewater. The tests were completed for 2 and 20h
18 for the phosphorus release and denitrification tests, respectively. Samples of 60 ml were taken every
19 0.5h for the first 2h. The samples were analysed for VSS, SCOD, $\text{PO}_4\text{-P}$ and $\text{NO}_3\text{-N}$. Phosphate
20 release and denitrification rates were calculated after 2h and 1.5h, respectively. Replicates of the P
21 release and denitrification tests were not completed because the wastewater and RAS needed to be
22 used within 1-2 hours after collection. Therefore the 4 series of experiments were completed in
23 batches and results compared [20].

24

25

26

1 2.4 Analytical methods

2 The samples were centrifuged at $8804\times g$ for 20 min (Hettich Zentrifugen, Tuttlingen, Germany)
3 and the supernatant filtered through a $0.45\ \mu\text{m}$ (glass-fiber filter paper) prior to analyses. Chemical
4 oxygen demand, $\text{NO}_3\text{-N}$, NH_4^+ , $\text{PO}_4\text{-P}$ were determined using Merck Spectroquant cell test
5 (Darmstadt, Denmark) according to the manufacturer instructions. Solids determination, TSS and
6 VSS, was performed according to Standard Methods [21]. All the analyses were completed in
7 duplicate.

8
9 For VFAs determination, 9 ml of the filtrate was placed in 10 ml plastic tubes and acidified with
10 $10\ \mu\text{l}$ of sulphuric acid (98% purity) and frozen until high performance liquid chromatography
11 (HPLC) analyses was completed. The VFAs samples were analysed in triplicate using a HPLC
12 (Shimadzu VP Series, Shimadzu, Milton Keynes, UK) with the ultraviolet (UV) detector set at
13 208 nm. The columns Biorad (cat. 125-0115) ($105\ \text{mm} \times 7.8\ \text{mm}$) and the guard column (Biorad
14 cat. 125-0131) were maintained at 65°C . The injection volume was $15\ \mu\text{l}$ and each sample was run
15 for 0.5h using 1 mM sulphuric acid pumped at a flow rate of $0.8\ \text{ml min}^{-1}$. A mixture of VFAs
16 (acetic acid, propionic acid, butyric acid and valeric acid analytical grade) at concentrations
17 between $0.05 - 1\ \text{g l}^{-1}$ was used as internal standard.

18 19 **3. Results and discussion**

20 *3.1 Soluble COD and VFAs production*

21 Soluble COD and VFAs concentrations of SAS increased to $3.6\ \text{g l}^{-1}$ and $0.37\ \text{g l}^{-1}$ after mechanical
22 disintegration by a deflaker corresponding to a 9 fold and 4.1 fold increases from initial values,
23 respectively (Table 2). The primary sludge was fermented during 80h and after this period the
24 SCOD increased to a maximum value of $10.3\ \text{g l}^{-1}$ (Site 5) with an average SCOD increase of 2 fold
25 for all sources of primary sludge fermented (Table 2). Likewise, the VFAs concentration increased
26 to an average value of $6.5\ \text{g l}^{-1}$ with the highest VFAs production observed for the sludge from Site

1 5 with a final concentration of 8.7 g l^{-1} . The maximum increase in VFAs was 2.5 fold from the
2 initial concentration (Table 2).

3

4 The VFAs and SCOD production yields from SAS disintegration were $6 \text{ g VFAs kg}^{-1} \text{ TSS}$ and 62 g
5 $\text{SCOD kg}^{-1} \text{ TSS}$. In comparison, the yields from primary sludge fermentation were $81 \text{ g VFAs kg}^{-1}$
6 TSS and $95 \text{ g SCOD kg}^{-1} \text{ TSS}$. The SCOD and VFAs yields indicate that primary sludge
7 fermentation promoted VFAs production while SAS disintegration mainly induced SCOD release.
8 Full-scale static primary sludge fermenter thickeners have yields of $25\text{-}40 \text{ g VFAs kg}^{-1} \text{ sludge}$ after
9 2-3 days [9], which are in the same order of magnitude of the yields described here.

10 For all types of sludge fermented (Sites 1 to 5) and disintegrated SAS the production of VFAs was
11 found to be linearly correlated with SCOD release (Fig. 1). The correlation factors (R^2) were
12 calculated over the 80h fermentation period and the values recorded were between 0.99 (Site 2) and
13 0.86 (Site 5) and therefore can be considered linearly correlated. Hence, VFAs were the main
14 SCOD constituent during fermentation independent of the initial sludge initial characteristics.
15 Fermentation of sludge is described the happen through hydrolysis of complex organic matter into
16 soluble organic compounds that are transformed into volatile fatty acids during acidogenesis.
17 Between 69-94% of the SCOD generated during primary sludge fermentation from sites 1-5 were
18 VFAs (Table 2, Fig.1). Others have reported a proportion of 85% VFAs [5]. Comparatively, only
19 approximately 12% of the SCOD released during SAS disintegration were VFAs. In a previous
20 study it was demonstrated that the SCOD released during SAS disintegration was composed of
21 proteins (30%), carbohydrates (13%) and VFAs (12%) but the remaining 45% of the SCOD was not
22 characterised [16]. Other studies have also identified SCOD as the main product [22] that could be
23 fractionated into proteins, extracellular polymeric substances and soluble microbial products after
24 ultrasound [23, 24] or thermal treatment [25], but the identification of the complete range of
25 substances that contribute to SCOD has not yet been described.

26

1 Mainly acetic acid (41%) and propionic acid (36%) were produced during primary sludge
2 fermentation for Sites 1, 2 and 3 with an acetic acid production of 47-62 g kg⁻¹ TSS (Fig. 2). Other
3 authors have observed equivalent VFAs production after fermentation of primary sludge at 20°C
4 with 43% of acetic acid and 41% of propionic acid [19]. VFAs production was observed to
5 predominantly occur during the first 40h of primary sludge fermentation with rates between 0.118 g
6 VFAs h⁻¹ (Site 1) to 0.188 g VFAs h⁻¹ (Site 5). Between 46h and 80h, fermentation processes
7 occurred at a lower rate and the VFAs formation rates decreased to values of 0.03 g VFAs h⁻¹ (Site
8 1). On the other hand, SAS disintegration mainly produced acetic acid (90%) and propionic acid
9 (10%) (Fig. 2).

10

11 3.2 Nutrient removal predictive tests

12 Nutrient predictive tests can be used to estimate the BNR performance using a specific carbon
13 source [20]. These tests are based on the PO₄-P release after a specific period of time and the
14 denitrification rates, giving an indication of P and N removal, respectively. Two tests were
15 conducted in order to match the carbon addition in VFAs (3.5 mg l⁻¹) and SCOD (50 mg l⁻¹)
16 concentration with the aim of assessing separately the influence of SCOD and VFAs on P and N
17 removal. The nutrient removal tests were done in parallel using the same source of wastewater
18 implying the same microbial community as inoculum and only parameter that was changed was the
19 carbon source. Therefore it should be possible to compare the impact of the different carbon sources
20 on the nutrient removal rates.

21

22 When the carbon addition was matched in total VFAs concentration, the highest P release rates
23 were obtained for the disintegrated SAS supernatant and disintegrated SAS with values of 0.026
24 and 0.025 mg PO₄-P g⁻¹ VSS min⁻¹, respectively (Fig. 3a). Comparable results were obtained in the
25 denitrification tests with the highest denitrification rates measured for the SAS disintegrated sludge
26 and supernatant 0.039 and 0.036 mg NO₃-N g⁻¹ VSS min⁻¹, respectively (Fig. 4a). When the carbon

1 addition was matched in terms of SCOD the highest phosphorus release rate was obtained for
2 disintegrated SAS ($0.031 \text{ mg PO}_4\text{-P g}^{-1} \text{ VSS min}^{-1}$) and the lowest for acetate ($0.024 \text{ mg PO}_4\text{-P g}^{-1}$
3 VSS min^{-1}) (Fig. 3a). The denitrification tests showed the same trend with rates of 0.038, 0.040 and
4 $0.039 \text{ mg NO}_3\text{-N g}^{-1} \text{ VSS min}^{-1}$ for disintegrated SAS, disintegrated SAS supernatant and fermented
5 primary sludge, and the lowest rate for acetate with $0.027 \text{ mg NO}_3\text{-N g}^{-1} \text{ VSS min}^{-1}$ (Fig. 4a).
6 From the nutrient predictive tests it can be established that the carbon obtained from the sludge was
7 more suitable for BNR (phosphate release and denitrification) than the external acetate carbon
8 source because primary sludge fermentation and disintegrated SAS gave higher phosphate releases
9 and denitrification rates. This observation is supported by Fig. 3b and 4b that show phosphate
10 releases and denitrification rates above the values in the control and acetate vessels (baseline).
11 Furthermore, the denitrifying bacteria seem to be able to use a wider range carbon sources as
12 identified through the comparison of rates when the carbon was matched in total VFAs and SCOD
13 (Figure 4b). Denitrification metabolism is favoured over phosphate release because denitrifying
14 bacteria compete over readily available carbon sources with PAOs and can use wider type of carbon
15 [26]. The denitrification rates recorded ($1.4\text{-}1.9 \text{ mg NO}_3\text{-N g}^{-1} \text{ VSS h}^{-1}$) were similar to those
16 obtained with ozonated SAS as a carbon source [29] (Table 3). Denitrification rates have been
17 demonstrated to be dependent on the biomass concentration [30] but also the origin of the biological
18 material and its activity [31] which could possibly explain the high rates obtain in other studies that
19 varied between $7\text{-}41 \text{ mg NO}_3\text{-N g}^{-1} \text{ VSS h}^{-1}$ (Table 3). This emphasizes that denitrification tests
20 should be completed for a specific wastewater/carbon source and compared with a control using the
21 same source of denitrifying bacteria [20]. Treatment of ammonia and nitrate in anaerobic
22 conditions has also been demonstrated by using enriched cultures of ANAMMOX (anaerobic
23 ammonium oxidation bacteria) [27].

24

25

1 The phosphorus release rates ($0.9\text{-}1.5\text{ mg PO}_4\text{-P g}^{-1}\text{ VSS h}^{-1}$) determined for the internal carbon
2 sources were in the same range of reported rates, between $1.3\text{-}2.5\text{ mg PO}_4\text{-P g}^{-1}\text{ VSS h}^{-1}$ (Table 3).
3 The PAOs responsible for phosphate release, were more selective with regard to carbon source use
4 as was demonstrated by differences in the $\text{PO}_4\text{-P}$ release rates when the carbon source was matched
5 in total VFAs in comparison with SCOD (up to $0.01\text{ mg PO}_4\text{-P g}^{-1}\text{ VSS min}^{-1}$ higher when matched
6 in total VFAs) (Fig. 3b). The internal carbon sources not only contained VFAs, but also other
7 substances that contributed to the SCOD. The SCOD of the wastewater after internal carbon
8 addition matched on VFAs was on average 63 mg l^{-1} (fermented primary sludge addition) and 91
9 mg l^{-1} for the disintegrated SAS in comparison with 50 mg l^{-1} in the vessel supplemented with
10 acetate. Hence, the microbial species that complete phosphate release not only use VFAs but also
11 other sources of soluble carbon [20]. Adaptation of the biomass to the carbon source could further
12 enhance the phosphorus release rates to $5.9\text{ mg PO}_4\text{-P g}^{-1}\text{ VSS h}^{-1}$ as it has been demonstrated by
13 Puig et al. [28].

15 3.3 Carbon to nutrient ratios

16 The fermented primary sludge and the disintegrated sludge were further compared by calculating
17 the carbon to phosphorus ($\text{SCOD:PO}_4\text{-P}_{\text{release}}$) and carbon to nitrate ($\text{SCOD:NO}_3\text{-N}$) ratios which
18 can be used to estimate the BNR potential of a specific wastewater/carbon source [2] (Table 3). The
19 $\text{SCOD:PO}_4\text{-P}_{\text{release}}$ ratio varied between 8:1 for disintegrated SAS and 15:1 for acetate and the
20 $\text{SCOD:NO}_3\text{-N}$ ratio ranged between 9:1 and 12:1 (Table 3). Both $\text{SCOD:PO}_4\text{-P}_{\text{release}}$ and SCOD:
21 $\text{NO}_3\text{-N}$ ratios indicate that the most effective carbon source for BNR was disintegrated SAS. A
22 substantial difference between $\text{SCOD:PO}_4\text{-P}_{\text{release}}$ for disintegrated SAS and fermented primary
23 sludge with values of 8 and 15, respectively, was observed. As previously described, nutrient
24 release with disintegrated SAS was not enhanced by the presence of viable PAOs after
25 disintegration [20]. The difference between $\text{SCOD:PO}_4\text{-P}_{\text{release}}$ ratio recorded for the SAS and
26 primary sludge carbon sources was likely to be linked to the type of carbon and bioavailability. The

1 main SCOD component of fermented primary sludge was VFAs corresponding to 90% of the
2 SCOD and this type of carbon source has been extensively studied for BNR enhancement [2, 7, 8].
3 Yet the SCOD products of SAS disintegration have not been completely identified since only
4 approximately 12% were VFAs with 45% of the SCOD remaining uncharacterised [16]. Further
5 work needs to be completed to identify the products of the SAS disintegration that enhance BNR (P
6 release and NO_3^- reduction) at rates above acetate and fermented primary sludge (Table 3).

7 8 *3.4 Implications of using primary sludge fermentation and SAS disintegration as carbon sources to* 9 *the sewage treatment works*

10 The disintegration of SAS was observed to enhance NH_4^+ and $\text{PO}_4\text{-P}$ concentrations from 10 mg
11 $\text{NH}_4^+ \text{ l}^{-1}$ and 159 mg $\text{PO}_4\text{-P l}^{-1}$ to 60 mg $\text{NH}_4^+ \text{ l}^{-1}$ and 500 mg $\text{PO}_4\text{-P l}^{-1}$, respectively, i.e. the nutrients
12 increased by a factor of 6 (NH_4^+) and 3.5 ($\text{PO}_4\text{-P}$) (Table 2). The SAS disintegration promoted
13 higher phosphate release than sludge fermentation since this nutrient is removed from the
14 wastewater by accumulation in the biomass [32] and it was solubilised during the disintegration
15 process. The NH_4^+ and $\text{PO}_4\text{-P}$ concentrations were also observed to increase during the
16 fermentation period (Table 2) and a strong correlation was observed between acetic acid production
17 with both ammonia (correlation factors R^2 from 0.73 to 0.96) and phosphorus release (correlation
18 factors R^2 from 0.88 to 0.94). On average, the $\text{PO}_4\text{-P}$ and NH_4^+ release from the fermentation of
19 primary sludge was 2.1 and 4 fold higher than the initial concentration of 18.3 mg $\text{PO}_4\text{-P l}^{-1}$ and
20 111.9 mg $\text{NH}_4^+ \text{ l}^{-1}$.

21 The implications of using primary sludge fermentation and SAS disintegration as carbon source
22 were assessed by completing a nutrient mass balance to a full-scale BNR treatment works treating
23 10,000 $\text{m}^3 \text{ day}^{-1}$ of wastewater (Table 4). The volumes of fermented primary sludge and
24 disintegrated SAS added to the BNR settled sewage were calculated based on predictive tests match
25 on SCOD (fermented primary sludge 4.2 ml in 2 l of wastewater and disintegrated SAS 13 ml in 2 l
26 of wastewater) with dilution factors of 0.0021 and 0.0065 in the wastewater, respectively. The

1 addition of fermented sludge would increase the ammonia load by 13 kg day^{-1} and $\text{PO}_4\text{-P}$ by 2 kg
2 day^{-1} in the settled sewage fed to the BNR process. The addition of disintegrated SAS was likely to
3 increase the $\text{PO}_4\text{-P}$ in the settled sewage feeding the BNR process by an additional load of 33 kg
4 day^{-1} .

5
6 The ratios of nutrient recycled to the BNR versus SCOD feed to the process indicate that the use of
7 disintegrated SAS as internal carbon source could lead to a cycling of phosphorus in the STW with
8 $0.97 \text{ g PO}_4\text{-P kg}^{-1} \text{ SCOD}$. Ammonia concentrations would also be increased ($0.11 \text{ g NH}_4^+ \text{ kg}^{-1}$
9 SCOD) but to a lesser extent than for phosphorus (Table 4). The addition of primary sludge
10 fermentation products for enhancing the BNR process is recommended since the recycling of
11 nutrients would be minimised ($0.02 \text{ g PO}_4\text{-P kg}^{-1} \text{ SCOD}$ and $0.13 \text{ g NH}_4^+ \text{ kg}^{-1} \text{ SCOD}$). For the SAS
12 disintegration product to be a suitable carbon source for BNR and complement the beneficial carbon
13 to nutrient ratios here described, the phosphorus would potentially need to be removed from the
14 disintegration liquors. Phosphate removal could be achieved using chemical precipitation with iron
15 sulphate or alum or alternatively, struvite ($\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$) formation could be promoted by
16 providing suitable conditions for its precipitation (pH 8.5 and a ratio of 1:1:1 of
17 phosphate:ammonia: magnesium) with 80% phosphate recovery [33]. In addition, an economical
18 evaluation has demonstrated that sludge disintegration using a deflaker is an energy intensive
19 process (6138 kW day^{-1} in a $50,000 \text{ m}^3 \text{ day}^{-1}$ treatment works) [20] in comparison with sludge
20 fermentation with virtually no operational costs associated [9].

21

22 **4. Conclusions**

23 The fermentation of primary sludge promotes VFAs production as 69-94% of the SCOD generated
24 consisted of VFAs, compared with approximately 12% VFAs in the SCOD released during SAS
25 disintegration. Higher VFAs yields were observed during the first 40h of fermentation.

26

1 The nutrient removal tests demonstrated that internal carbon sources obtained from sewage sludge
2 enhanced phosphate release and denitrification compared with acetate. The disintegrated SAS was
3 the more suitable carbon source for BNR compared with fermented primary sludge (SCOD:PO₄-
4 P_{release} ratio of 8 and SCOD:NO₃ ratio of 9) since other components contributing to SCOD besides
5 VFAs promoted BNR. Further work should be completed to identify these products of SAS
6 disintegration. However for disintegrated SAS to be considered a suitable carbon source for BNR
7 the PO₄-P would potentially need to be removed before recycling to the BNR wastewater. Overall
8 the addition of primary sludge fermentation products for enhancing the BNR process is
9 recommended since the recycling of nutrients would be minimised (0.02 g PO₄-P kg⁻¹ SCOD and
10 0.13 g NH₄⁺ kg⁻¹ SCOD).

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19 **Figures captions:**

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21 **Fig. 1.** Correlation between SCOD and VFAs content in COD equivalent (VFAs-COD, calculated
22 according to Lie and Welander [38] that reported COD equivalent constants of 1.066 for acetic acid,
23 1.512 for propionic acid, 1.816 for butyric acid, and 2.036 for valeric acid), for Site 1 (\diamond); Site 2
24 (\square), Site 3 (\triangle), Site 4 (\circ), Site 5 (\times) and SAS sludge (\bullet). The correlation coefficients (R^2) for
25 the curves varied between 0.86 for Site 4 and 0.99 for Site 2.

26

1 **Fig. 2.** VFAs concentrations after 80 hours of fermenting the primary sludge collected from 5
2 different full-scale sites and after mechanical disintegrating SAS originated from a pilot-scale BNR
3 reactor with a deflaker: acetic acid (□), propionic acid (■), butyric acid (▨) and valeric acid (▩).
4 The error bars show the standard deviation of the duplicate fermenters.

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7 **Fig. 3.** a - Phosphorus release rate when the carbon addition was matched in total VFAs (□) and
8 SCOD (■) in the control vessel with no addition of carbon source (1); with acetate (2); with
9 fermented primary sludge from Site 1 (3), with disintegrated SAS (4) and with disintegrated SAS
10 supernatant (5). b – Calculated phosphorus release rate above control vessel (with no addition of
11 carbon source) and acetate supplemented vessels.

12
13 **Fig. 4.** a - Denitrification rate when the carbon addition was matched in total VFAs (□) and SCOD
14 (■) in the control vessel with no addition of carbon source (1); with acetate (2); with fermented
15 primary sludge from Site 1 (3), with disintegrated SAS (4) and with disintegrated SAS supernatant
16 (5). b – Calculated denitrification rate above control vessel (with no addition of carbon source) and
17 acetate supplemented vessels.

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Table 1. Carbon added to the phosphorus release test and denitrification test when the carbon source was matched in SCOD or VFAs.

	Carbon match (mg l⁻¹)	Initial SCOD (mg l⁻¹)	Vessel 1 (Control)	Vessel 2 (Acetate) (ml)	Vessel 3 Primary sludge supernatant (ml)	Vessel 4 Disinteg. SAS (ml)	Vessel 5 Disinteg. SAS supernatant (ml)
P test	VFAs: 3.5	50	-	2.5	7.4	25.0	25.0
	SCOD: 50	40	-	5.0	4.2	12.0	13.0
N test	VFAs: 3.5	49		1.2	3.6	32.0	32.0
	SCOD: 50	34		5.8	4.9	15.0	15.0

Table 2. Primary sludge and SAS composition before and after 80h fermentation at 20°C and disintegration with deflaker.

Primary sludge	Before fermentation				After fermentation			
	SCOD (g l ⁻¹)	NH ₄ (mg l ⁻¹)	P (mg l ⁻¹)	VFAs (g l ⁻¹)	SCOD (g l ⁻¹)	NH ₄ (mg l ⁻¹)	P (mg l ⁻¹)	VFAs (g l ⁻¹)
Site 1	5.6	187.0	50.3	2.2	10.2	598.0	87.5	6.3
Site 2	4.8	70.2	3.5	3.3	7.5	543.0	6.6	5.2
Site 3	2.3	128.0	10.7	2.9	7.8	307.7	22.8	4.6
Site 4	4.0	70.2	23.5	2.4	7.5	300.0	42.5	5.4
Site 5	4.1	104.0	3.5	3.5	10.3	200.0	11.6	8.7
SAS	Before disintegration				After disintegration			
Pilot-scale BNR	0.4	10.0	159.0	0.09	3.6	60.0	500.0	0.37

The standard deviation obtained for the duplicate fermentations was: 0.5 g l⁻¹ for SCOD; 3.0 mg l⁻¹ for NH₄⁺; 1.0 mg l⁻¹ P and 0.5 g l⁻¹ for VFAs.

Table 3. Carbon to nutrient ratios and phosphorus release rates and denitrification rates over a range of internal and external carbon sources.

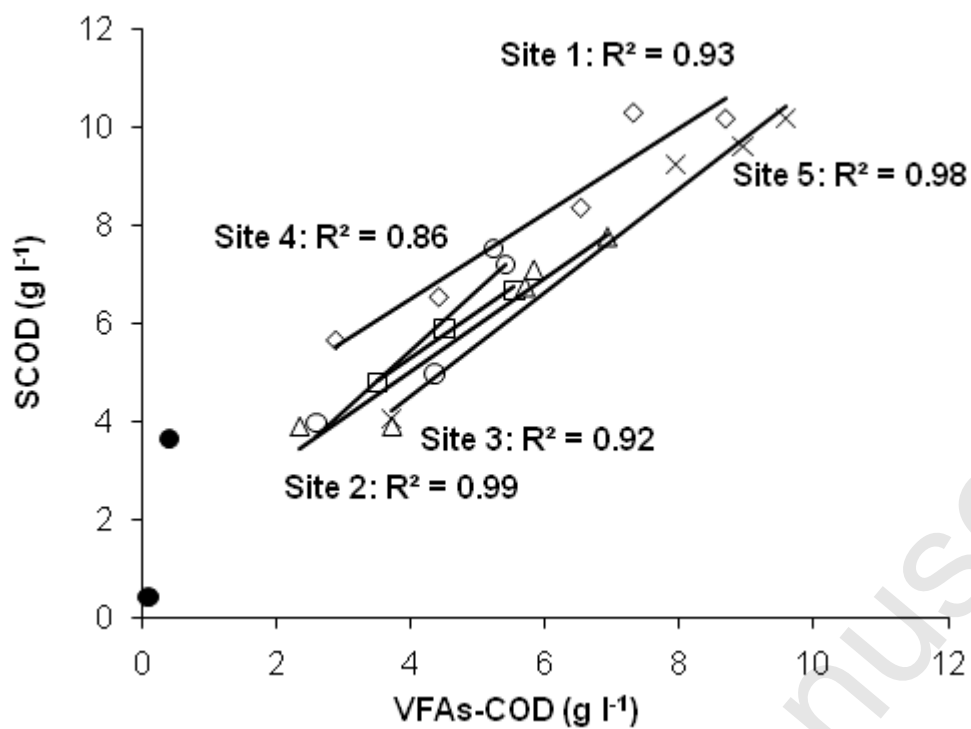
Carbon source	PO ₄ -P			NO ₃ -N		
	SCOD: PO ₄ -P	mg PO ₄ -P g ⁻¹ VSS h ⁻¹	Reference	SCOD: NO ₃ -N	mg NO ₃ -N g ⁻¹ VSS h ⁻¹	Reference
Acetate Fermented primary sludge	15.0	0.5	This study	9.0	0.9	This study
Disinteg. SAS	8.0	1.5	This study	9.0	1.9	This study
Disinteg. SAS supernatant*	9.0	1.4	This study	12.0	1.6	This study
Various substrates	4.9-10.0		[34]			
Acetate (30 adapted)		5.9	[28]			
Ethanol (non-adapted)**		1.5	[28]			
Hydrolysed primary sludge					41.0	[35]
Mechanically disintegrated SAS					15.0	[36]
Ozonated SAS					0.5-3.4	[29]
Sucrose (biomass in immobilised filter)				2.5		[30]
Acetate				10.0		[37]
Acetate Disintegrated SAS		1.3	[20]		7.0	[20]
		2.5	[20]		15.0	[20]

* Disintegrated SAS supernatant was obtained after centrifuging disintegrated SAS and therefore it was mainly composed of soluble products.

**Microbial community used was not pre-adapted to ethanol as single carbon source

Table 4. Phosphate and ammonia mass balance on a BNR treatment plant with a capacity of 10,000 m³ day⁻¹ after addition of fermented primary sludge or disintegrated SAS supernatant.

		Primary sludge supernatant	Disinteg. SAS supernatant
Dilution factor calculated from the nutrient removal predictive tests		0.0021 (4.2 ml in 2 l of wastewater)	0.0065 (13.0 ml in 2 l of wastewater)
Nutrients in the sludge	PO ₄ -P (mg l ⁻¹)	87	500
	NH ₄ ⁺ (mg l ⁻¹)	598	60
Increased nutrient load to the BNR process	PO ₄ -P (kg day ⁻¹)	2	33
	NH ₄ ⁺ (kg day ⁻¹)	13	4
Ratio between nutrient recycled and SCOD	g PO ₄ -P kg ⁻¹ SCOD	0.02	0.97
	g NH ₄ ⁺ kg ⁻¹ SCOD	0.13	0.11



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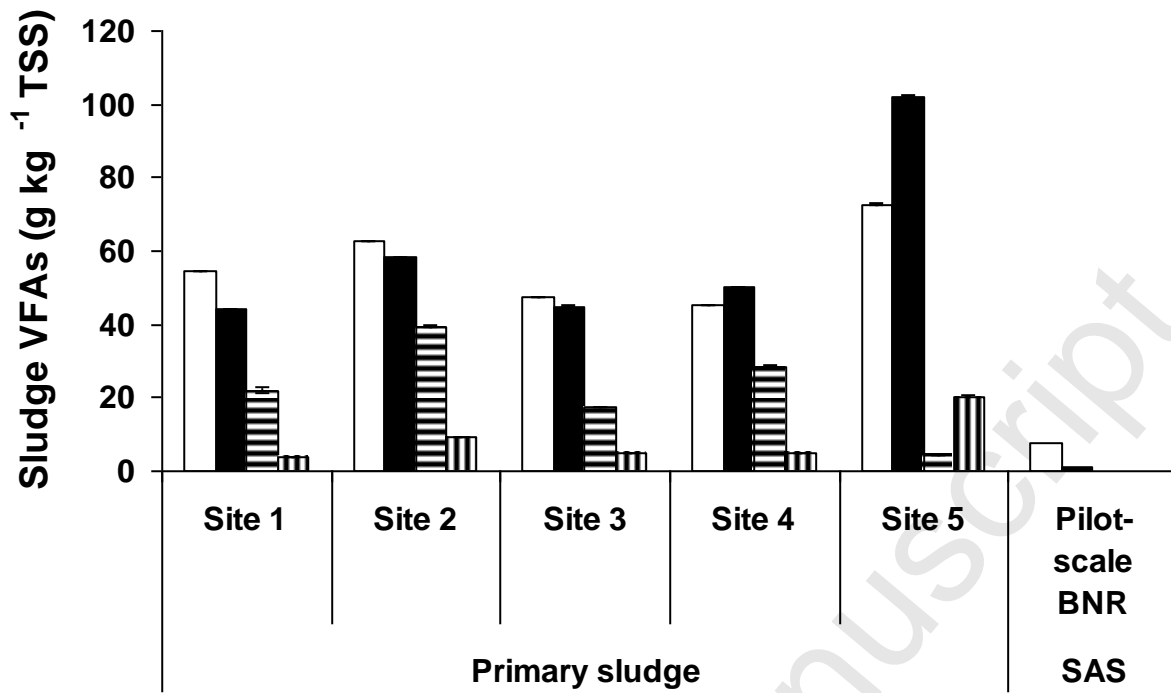
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4 Fig. 2.

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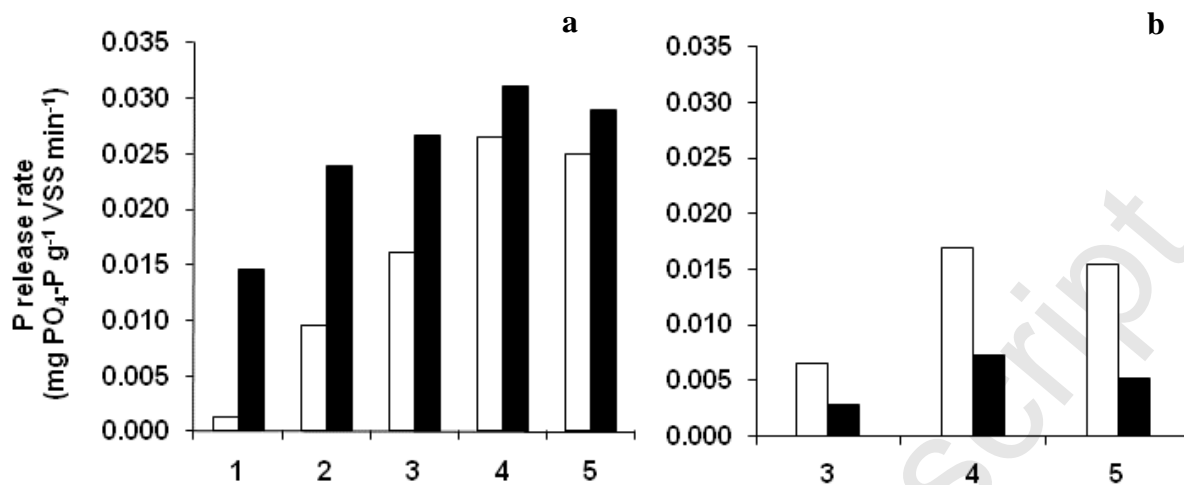


Fig. 3.

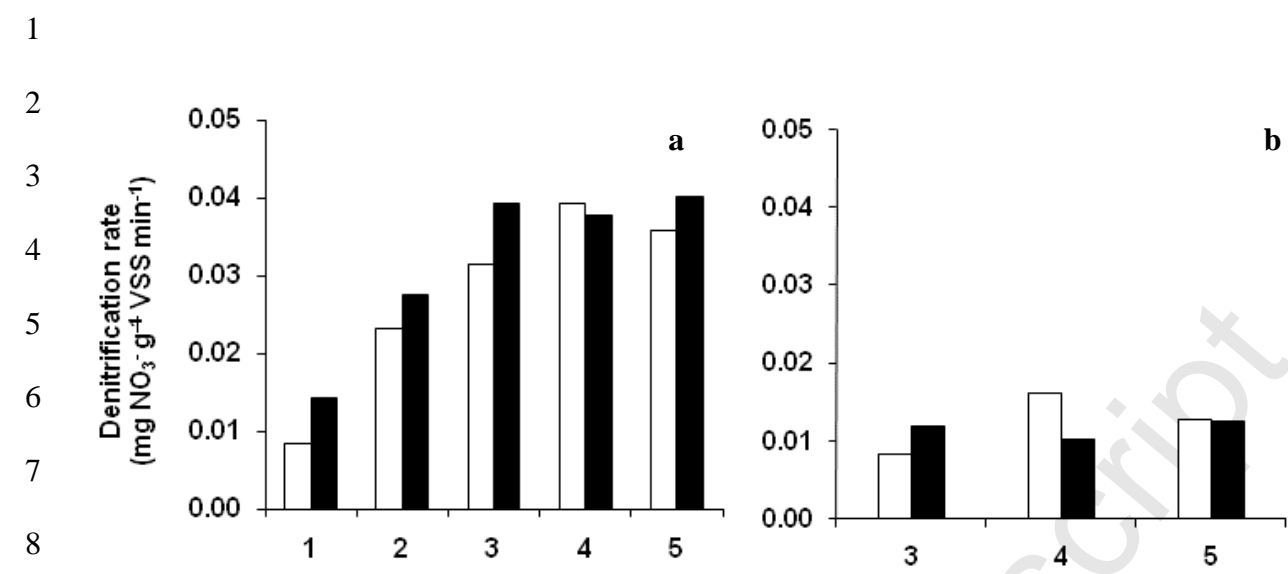


Fig. 4.