Mine sludge waste recycling as bio-stimulant for applications in anaerobic wastewater treatment

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ABSTRACT

This study examined the applicability of two mine sludge wastes, mine tailing sludge (MTS) and acid mine drainage sludge (AMDS) as iron-rich bio-stimulant for enhancing organic matter degradation in anaerobic process. Batch treatment of domestic sewage having 343 ± 10 mg/L chemical oxygen demand (COD) using MTS and AMDS as additives mixed with septic tank sludge as anaerobic inoculum produced lower start-up time, higher efficiency of COD removal, enhanced biomass retention, and higher acidogenic and methanogenic activity after stabilization. Biostimulation induced by mine sludge waste additives in anaerobic system were observed to have correlation with percentage of iron content in the additives, as well as difference in surface charge between biomass and the additives. Treatment efficiency induced by the two mine sludge waste based additives were similar at 90% confidence limit, however, was found to be higher than lower iron containing additive laterite soil, while lower than higher iron containing synthetic zero valent nano iron as additives used for comparison. The study was supported by scanning electron microscope, atomic force microscope and optical microscope images of sludge granule sand surface charge measurement.

Key words | biostimulation, COD removal, mine waste, start-up time, wastewater treatment

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INTRODUCTION

The mine sludge consisting of sludge from the tailings or settleable solids in the acid mine water (acid mine drainage) is perceived as a severe environmental burden and hence needs proper management. Typically, mine waste sludge in India, as well as in rest of the world, is disposed of in open pits or landfills (Rakotonimaro *et al.* 2017). Sludge generated from mine tailings are mostly rich in oxides of heavy metals iron and manganese in major along with zinc, copper, aluminum, nickel, etc., in minor quantities (Ghose & Sen 2001). Sludge generated from the neutralization of acid mine drainage water additionally contain calcium (in the form of gypsum) due to the use of quick lime as a neutralizing agent during treatment (Maree & Du Plessis 1994). Mine waste sludge dumped in landfills may pose a threat of leaching of heavy metals into the aquifer underneath. On the other hand, mine waste sludge dumped in open pits may produce run-off with rain and reach a nearby water body causing harm to the ecosystem. To address the issue of hazard associated with dumping mine waste, recently several reuse options of mine sludge have been explored, many of which are driven by the use of beneficial micronutrient constituents (iron, manganese, copper, zinc, etc.) present in the mine sludge. The major reuse applications of mine sludge waste include uses of mine sludge for metal recoveries, as an additive for making Portland cement, as micronutrients source in fertilizers and soil stabilizers, and as adsorbent in water purification (Rakotonimaro *et al.* 2017). Of these, use of mine sludge waste for water or wastewater treatment particularly looks more attractive from environmental management perspective, as it not only considers beneficial reuse of mine sludge, but also presents a low-cost alternative approach for reducing water pollution. However, the uses of mine sludge in water or wastewater treatment have been limited to small scale adsorption studies (Rakotonimaro *et al.* 2017) and their potential impacts on biodegradation of organic contaminants have largely been ignored.

The micronutrients, especially iron and manganese, have been reported to be essential cofactors in a number of biological pathways promoting growth and metabolism in microbial processes (Eltarahony *et al.* 2018). The use of these micronutrients as bio-stimulant has shown encouraging results, especially for enhancing hydrolysis and

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methanogenesis activities of microorganisms in anaerobic digestion processes (Qiang *et al.* 2013). Anaerobic treatment processes have gained popularity in wastewater treatment owing to its lower operation and maintenance cost, ease of operation and potential recovery of energy in the form of biogas; however, it suffers from longer start-up time (3–5 months) and relatively lower treatment efficiency compared to aerobic processes. It is known that stability and efficiency of an anaerobic process like upflow anaerobic sludge blanket (UASB) reactor depends upon extent of granulation of the sludge bed, which involves agglomeration of biomass ensuring higher retention of microorganism in the reactor (Tiwari *et al.* 2005). Various polymers and metals have been reported to induce lowering of granule formation time and increase in treatment efficiency (Yu *et al.* 2000). In particular, materials rich in iron in its natural form have been effective in enhancing microbial activity (Ranmadugala *et al.* 2017). For instance, oxide of iron (which is available in mine waste in major proportion), iron rich fly ash, and zero valent iron additives have been reported to induce biofilm formation, enhance methanogenic activity in anaerobic digestion, and improve bioremediation efficiency of organic contaminants (Bose *et al.* 2016; Montalvo *et al.* 2017; Ranmadugala *et al.* 2017). However, application of such chemical additives require chemical doses, thereby increasing the chemical footprint and the overall cost of the treatment process. Thus, it will be economically viable as well as environmentally sustainable if a waste material like mine sludge, which is rich in essential metal like iron in its natural form, could be effectively utilized as bio-stimulant supplement in anaerobic microbial processes.

The aim of the present study is to explore the role of two forms of mine sludge waste viz. mine tailing sludge (MTS) and acid mine drainage sludge (AMDS) as bio-stimulating agents and their effect on the efficiency of anaerobic treatment process. The comparisons with two other iron base supplements, zero valent nano iron (ZVNI) having higher iron content, and laterite soil (LS) having lower iron content, was made to benchmark the performance of the mine sludge based additives as biostimulant.

MATERIALS AND METHODS

Wastewater and inoculum source

Domestic sewage collected every morning (9–10 AM local time) from a sewage pump house inside Indian Institute of Technology (IIT) Kharagpur campus, was used as influent for feeding anaerobic batch reactors. The characteristic of the sewage is mentioned in Table 1. Anaerobic sludge collected from a septic tank inside IIT Kharagpur campus having pH 7.3, TSS 47.6 g/L, VSS 30.4 g/L, and TOC 135 mg/g TS was used as inoculum for the study.

Mine waste and other additives

MTS was collected from a mining site at Jharkhand, India, and was washed in distilled water followed by drying in

Table 1 | Characteristics of influent sewage and treated effluent from the reactors operated in steady state

Parameters	Concentration in sewage (influent)	Concentration in effluent from control	Concentration in effluent from reactors supplemented with additives			
			ZVNI	*MTS*	*AMDS*	*LS*
COD (mg/L)	343.6 ± 10.8	153.4 ± 15.5	52.1 ± 7.2	72.9 ± 9.2	79.2 ± 9.5	106.7 ± 12.6
pH	7.5 ± 0.5	6.8 ± 0.7	7.2 ± 0.4	7.3 ± 0.3	7.4 ± 0.4	7.3 ± 0.2
TDS (mg/L)	4,895 ± 105	730 ± 103	823 ± 187	912 ± 158	1,037 ± 114	940 ± 142
Alkalinity (mg/L of $CaCO_3$)	203 ± 88	277 ± 66	374 ± 45	433 ± 93	464 ± 89	289 ± 58
TSS (mg/L)	140 ± 14	32.05 ± 9.05	7.34 ± 2.84	8.05 ± 3.05	10.1 ± 0.52	15.23 ± 0.49
VSS (mg/L)	130 ± 5	30.3 ± 4	6.32 ± 0.7	7.3 ± 0.5	9.4 ± 0.89	13.52 ± 1.25
Acetate (mg/L)	20.94 ± 3.08	29 ± 6.12	43 ± 4.96	39 ± 5.17	37 ± 4.62	33.17 ± 4.97
Propionate (mg/L)	Not detected	Not detected	8.2 ± 1.2	6.5 ± 0.9	4.2 ± 0.88	Not detected
NO_3 – N (mg/L)	48 ± 2.1	38.1 ± 4.9	30.3 ± 2.6	33.2 ± 4.5	34.5 ± 5.2	34.7 ± 5.3
NH_4^+ - N (mg/L)	6.1 ± 2.3	20.33 ± 4.88	28.3 ± 4.6	25.61 ± 4.2	26.17 ± 4.5	22.5 ± 3.73
TN (mg/L)	63 ± 7.2	63.13 ± 5.79	61 ± 3.61	62 ± 5.36	61.42 ± 3.5	62.66 ± 4.71
Methane (mL/g COD)		7	15	12	12	9

hot air oven for 24 h before using it for the study. LS was collected from an agricultural field inside IIT Kharagpur and was processed similar to MTS. AMDS was prepared in the laboratory by neutralizing synthetic acid mine drainage water according to the method suggested by Cocos *et al.* (2002). Chemicals used for synthesis of synthetic acid mine drainage water were $FeSO_4.7H_2O$ (3,971 mg/L), $CuSO_4$ (0.2 mg/L), $NiSO_4.6H_2O$ (3.5 mg/L), $CoSO_4.7H_2O$ (4.2 mg/L), $MnSO_4.H_2O$ (144.5 mg/L), $ZnSO_4.7H_2O$ (32 mg/L) and Na_2SO_4 (1,093 mg/L), pH was adjusted to 3 using a few drops of 1 N HCl and 1 N NaOH. Synthesized acid mine drainage water was neutralized by adding $CaCO_3$ under constant stirring condition, and content was centrifuged. Settled sludge (AMDS) was collected, washed in distilled water, oven dried for 24 h before use. ZVNI was synthesized according to the method suggested by Zhao & He (2011) by reducing $FeCl_3$ solution by $NaBH_4$. All the chemicals used were of purity ≥99% procured from Merck.

Experimental design

In a series of 500 mL conical flasks, pre-homogenized mixture of 5 g of a additives materials (viz. ZVNI, MTS, AMDS or LS) and 1.5 g of anaerobic inoculum sludge (on dry weight basis) were added to 300 mL of freshly collected domestic sewage. This resulted in 16.7 g/L concentration for additive materials and 5,000 mg/L MLVSS in the flasks. One flask was set as blank containing only inoculum and sewage, without any additive. Mouth of the flasks were plugged with cork and sealed with teflon tape. Each flask corresponded to an individual anaerobic bioreactor, and were subjected to a constant shaking at 120 rpm at 35 °C for 4 hours. The reactors were left to attain anaerobic condition naturally without the assistance of nitrogen purging. Anaerobic condition in the reactors were monitored before and after each set of experiment with the help of dissolved oxygen (DO) and oxidation – reduction potential (ORP) measurements. The ORP values in the range −100 to −350 mV indicated anaerobic conditions in the reactors (Vongvichiankul *et al.* 2017). The study was accompanied by a set of adsorptive control containing each additive materials in individual sterilized flasks but no biomass seed. Similarly one biosorptive control was also set using autoclave sterilized inoculum biomass. All other conditions for controls were kept similar. After 4 hours, shaking was stopped and the solid contents were allowed to settle for 1 hour. Supernatants were decanted and part of it was analyzed for TSS and VSS. The remaining supernatant was filtered under vacuum using 0.45 μm nylon filter then subjected to various other analysis. The 4 h study was conducted once a day and the settled sediment (containing inoculum and additive) was kept undisturbed inside the same conical flask with mouths sealed with cork and Teflon tape. After 19 h on the subsequent day, same experiment was repeated with 4 hour of shaking and 1 hour of settling followed by various analysis. The study was conducted continuously for 21 days. At the end of 21 days, all the solid contents (additives and biomass) were separated and characterized for selected physicochemical properties.

Analysis of treatment parameters

Elemental composition profile of all the additive materials were determined using X-ray fluorescence spectrophotometer (PANalytical) under normalization factor being 1.699 and minimum flow rate of helium being 0.64 L/min. Surface morphology was observed under scanning electron microscope (SEM) (Merlin), surface profile studied using atomic force microscope (AFM) (Agilent) and surface charge was determined using zetasizer (Malvern). Particle size of the additive materials were determined using hydrometer method (Bouyoucos 1962), while granule size distribution of the microbial inoculum and sludge were obtained by image analysis of images captured under optical microscope connected to high resolution camera (Leica).

Dissolved oxygen (DO), pH, oxidation reduction potential (ORP), and total dissolved solids (TDS) were measured using multiparameter device (YSI pro plus). Chemical oxygen demand (COD), volatile suspended solids (VSS), total suspended solids (TSS), total nitrogen (TN), nitrate nitrogen (NO_3^--N), ammonia nitrogen (NH_4^+-N), alkalinity and iron concentration were determined according to *Standard Methods* (APHA 1998). Total organic carbon (TOC) of inoculum sludge was determined using TOC analyzer (OI Analytical Aurora 1030 W). Soluble carbohydrate and protein in extracellular polymeric substances (EPS) in the inoculum sludge were determined calorimetrically using spectrophotometer (Orbit) by treating the sludge with Iron (III) chloride and sodium persulfate for 2 h and shaking at a constant speed of 150 rpm followed by centrifugation at 4,000 rpm (=8,944 × *g*) (Shi *et al.* 2015). Total carbohydrate was analyzed using phenol–sulfuric acid method (Nielsen 2010), while protein were analyzed using Bradford assay (Kruger 2002). Individual components of organic acids in volatile fatty acids (VFA) were analyzed using gas chromatography (Thermo scientific) using flame ionization detector, TG-1701MS column with nitrogen as career gas with flow rate 1.5 mL/min, split ratio 15 in auto-injector, ramping temperature 50 °C to 220 °C with

30 °C/min rise. Methane content in biogas generated from the reactors were determined using the downward displacement of water method wherein the gas outlet of the reactor was connected to an inverted infilled measuring cylinder placed over 200 mL 3% NaOH filled beaker. Since the reactors were run on lower organic load, the generated biogas after CO_2 absorption in 3% NaOH was presumed to contain over 95% methane (Tippayawong & Thanompongchart 2010).

RESULTS AND DISCUSSION

Characteristics of mine sludge waste and other additives

The four additives used in this study represented varying iron content as 92.5% in ZVNI, 64.1% in MTS used, 49.7% in AMDS, and 18.5% in LS as determined by X-ray fluorescence spectrophotometer (Table 2). Manganese was the other major element observed in 1.0, 15.3, 12.5 and 4.1% in ZNVE, MTS, AMDS and LS, respectively. Various other micronutrients such as Cu, Zn were available in <4% in all additives except ZVNI (Table 2). Particle size, specific gravity and surface roughness of the additives are also mentioned in Table 2. SEM images of MTS revealed spongy and porous structure with uneven flakes spread over, while SEM micrograph of AMDS revealed mesh like structure with inner surface of the mesh looking spongy and highly porous whereas outer edges of the mesh had uneven surface with mostly flake-like appearance (Figure 1(a) and 1(b)). Sponge-like inner surface is typically assumed to be composed of oxide of iron, whereas the flakes were presumably composites of silica, calcium oxide and

Table 2 | Physicochemical characteristics of additives used in the study

	Additives type			
Parameters	***ZVNI***	***MTS***	***AMDS***	***LS***
Surface charge (mV)	−10	−8	−5	−15
Roughness	0.41	0.47	0.55	0.77
Average size (nm)	200	243	250	350
Specific gravity	1.56	1.77	1.62	1.98
Iron (%)	92.5	64.1	49.7	18.5
Manganese (%)	1	15.3	12.5	4.1
Other elements (%)	B (5.5), Ni (1)	Si (6.5), Ca (4.8), Mo (0.2), Co (1.3), Ba (0.1), Al (2.5), Pb (0.3), Ni (3.2), Cu (0.5), As (0.6), Cr (0.1),Zn (0.45), Cd (0.1)	Ca (26.2), Co (2.3), Ni (1.7), Cu (3.9), Zn (3.7)	Si (60.3), Ca (3.3), Mo (1.2), Co (0.8), V (0.3), Se (0.22), Al (3.1), Pb (1.7), Ni (1.1), Cu (1.6), As (0.4), Cr (0.8), Zn (1.1), Cd (0.1)

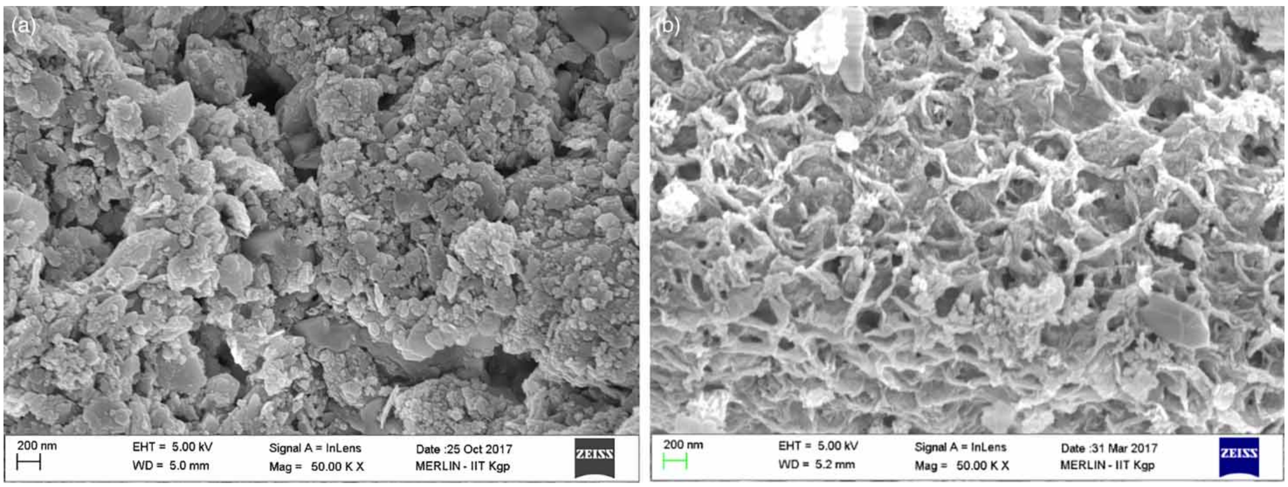

Figure 1 | Scanning electron microscope (SEM) images at 50 KX resolution of (a) mine tailing sludge showing porous irregular structure with uneven flake deposition and (b) acid mine drainage sludge showing porous structure with mess like flake deposition.

calcium sulfate. Porous and spongy structure of iron oxide particles were in agreement with the literature (Hallam *et al.* 2012).

Reactor start-up and onset of steady state

Only little removal in COD (<12%) was noticed in all the reactors during 4 h batch cycles in first 3 days, indicating microbial acclimatization during this lag phase. A spurt in COD removal efficiency was noticed post day 3 in all the reactors and the difference in COD removal efficiency between the reactors were distinctly noticeable thereafter (Figure 2(a)). The reactor supplemented with ZVNI was quickest to achieve steady state in 9 days, followed by MTS and AMDS supplemented reactors (10 days). The reactor supplemented with LS attained steady-state on the 15th day, while control was the slowest to attain stability in 18 days (Figure 2(a)). Further, it was observed that the acclimatization time inversely correlated with iron content in the additives, while the other analyzed properties of additives did not show any correlation with the time

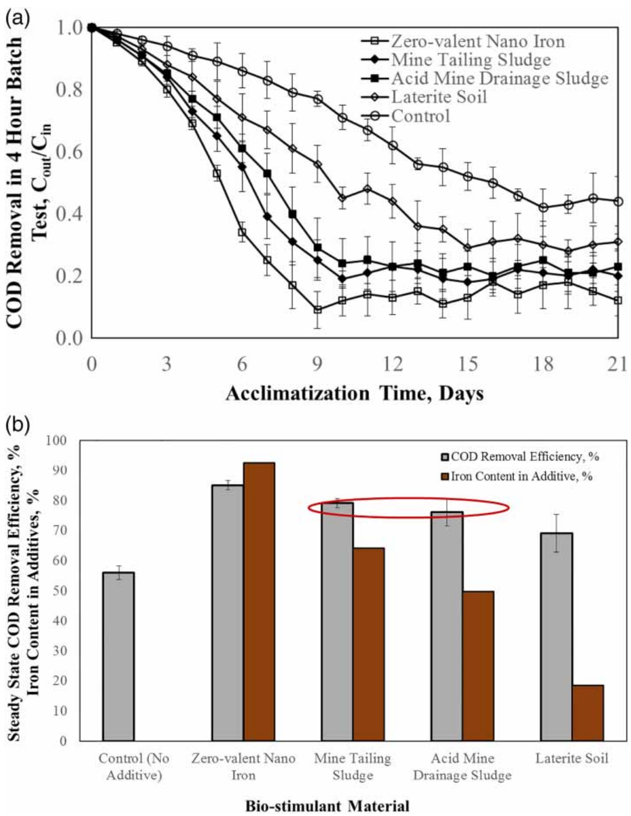

Figure 2 | COD removal in 4 h detention time in anaerobic batch studies in the presence of various iron rich bio-stimulant additives used during (a) 21-day steady period including acclimatization cycle and (b) steady state reactor performance after acclimatization along with iron content in the additives.

required to achieve steady state. Significant quantity of iron (up to 5 mg/L) leached during the first 3 days from various reactors supplemented with additives; however, no iron was detected in the effluent of any reactor during steady state. Post achieving the steady state all the reactors did not show any significant variation in COD removal or other effluent parameters measured confirmed with 95% confidence limit *F-test*. This indicated that the additives could be effective in reducing anaerobic reactors start-up time, thereby expediting steady-state appearance.

Steady state performance

The mine sludge additives, MTS and AMDS showed higher degree of COD removal, at around 77–79% compared to the 56% COD removal in controls (Figure 2(b)). The COD removal was primarily the result of microbial degradation, as adsorptive control showed negligible (<4%) COD reduction. The *F-test* indicated that post acclimatization, COD removal was not significantly different (at 90% confidence limit) for the two used sources of mine sludge. Although mine sludge additives were less effective than ZVNI which showed nearly 85% COD removal, they showed better COD removal than LS supplements (Figure 2(b)). The results suggested that the degree of COD reduction increased with increasing iron content in the additives (except between MTS and AMDS) (Figure 2(b)). The COD removal efficiency correlated linearly with goodness of fit (r^2) value of 0.93. The flow cytometry also confirmed the viability of cells to perfectly correlate with the percentage of iron present in the system under anoxic condition. Under anoxic conditions several previous studies have reported electron accepting property of iron oxides in biological reactions leading to enhancement in metabolism of microorganism present in vicinity (Ilbert & Bonnefoy 2013). Iron supplemented in microbial processes have been reported to produce higher growth, metabolism and metabolites formation as observed here. Presence of other metals like manganese, copper, nickel and zinc were not observed to produce any noticeable significant impact on the efficiency of COD removal or other parameters, as dominant proportion of iron content in all the additives appears to have shielded the effect of other metals (Montalvo *et al.* 2017).

Acetate was observed to be the dominant VFA species, while higher order VFA viz. butyrate, isobutyrate, valerate, etc., were not observed (Table 1). Concentrations of acetate and propionate varied with the iron content in the additives, except propionate was not observed in LS and control rectors. VFA are produced by acidogens and acetogens which are rapid growing species in any anaerobic process. In anaerobic systems, most of the biodegradable organics are first transformed to VFA by rapid growing acidogens and acetogens, before the methanogens initiate conversion of VFA into biogas, helping to prevent accumulation of VFA in the system which could decrease the pH and lowers down the efficiency of treatment. Reactor supplemented with ZVNI displayed highest methane generation, followed by MTS and AMDS, both showing equal rate of methane production (Table 1). Although higher than control, LS had the least methane production among reactors supplemented with additives (Table 1). Expectedly, the methane production correlated well with COD removal efficiency ($r^2 = 0.94$), however, it also showed a good linear correlation ($r^2 = 0.98$), with iron content in the additives. Iron containing additives like fly-ash have earlier been used to promote methanogenesis, however, the effect of varying iron content in additives on the process and extent of methanogenesis is little understood (Montalvo *et al.* 2017). The enhanced production of VFA as well as methane in the present study, indicated that iron rich additives have the potential to influence both acidogenesis and methanogenesis together leading to higher combined efficiency of anaerobic treatment process.

Alkalinity is an essential parameter in anaerobic treatment processes as it neutralizes the effect of decrease in pH due to accumulation of VFA in the system and helps promote methanogenesis. Increase in alkalinity in all the reactors was observed, presumably promoted by the generation of bicarbonate (HCO_3^-) and ammonium ion (NH_4^+) (Table 1). Ammonia nitrogen was observed to rise in all the reactors but were significantly lower than inhibition concentration (Chen *et al.* 2008). Highest alkalinity was recorded in the reactor supplemented with AMDS due to leaching of $CaCO_3$ from its surface, which was used as an neutralizing agent during acid mine water treatment. VFA to alkalinity ratio for all the reactors were observed to be in range 0.1–0.15, representing stable condition for methanogenesis (Ciotola *et al.* 2014). TDS in reactor supplemented with MTS, AMDS and LS was also contributed by leaching of Ca^{2+} ions, which present in significant proportion in these additives (Table 2). Irrespective of the presence of additive, pH in all the reactors remained in the range 6.8–7.4 throughout the study. Low value of ORP was observed in all the reactors, signifying prevailing sustained anaerobic and reducing conditions inside. ORP was observed to be the lowest in ZVNI supplemented reactor, i.e. −350 mV throughout the 21-day study. Low ORP in ZVNI supplemented reactor was probably due to continuous oxidation of the outer surface of ZVNI particles forming iron oxide layer and resulting in the creation of reducing condition in the surroundings. This could also be one of the reasons for higher COD removal efficiency in reactor supplemented with ZVNI, as higher reducing environment induces activity of obligate anaerobes like methanogens (Romero-Güiza *et al.* 2016). The rest of the reactors were observed to show ORP in the range of −100 to −250 mV during the entire duration of study and were not observed to follow any trend with the composition or surface charge phenomenon of the additives. Mass balance of carbon based on the steady state data showed that effluent VFA and biogas were the major routes of carbon mass outflow from ZVNI, MTS and AMDS supplemented reactors, whereas effluent COD and VFA were more prominent routes of carbon outflow from control, as well as LS supplemented reactor (Table 1). The contribution of effluent alkalinity was relatively low for all cases including control (Table 1). The unaccounted carbon mass was <5.5% in all the reactors.

Granulation and biomass retention

The solids in the effluent from all the reactors (measured in the supernatant after 1 h settling) were predominantly volatile as reflected by VSS/TSS ratio 0.90 ± 0.04. VSS, primarily composed of freely suspended microorganisms unable to attach with the additives, was the lowest in the reactor supplemented with ZVNI followed by MTS, AMDS, and LS, respectively, while VSS in the control was the highest (Table 1). This indicated the better retention of biomass in reactors supplemented with iron-rich additives. The surface charge of pure biomass (from control rectors) was observed to be −20 mV, whereas all the additives had relatively higher surface charges ranging from −5 to −15 mV (Table 2). The difference in surface charge induced partial positive charge on the surface of the additives material leading to the adhesion of microbes on to the surface of the additives thereby reducing the VSS in the effluent. Similar effect of surface charge of bacteria adhesion was reported recently (Yuan *et al.* 2017).

All the reactors were started with similar initial biomass as 5,000 mg/L, however, significant biomass loss took over with decanted effluent during unstable startup-phase, leading to a reduction in biomass levels in all the reactors. The biomass washout reduced to low levels as reactors approached steady state. At the end of 21 days, ZVNI supplemented reactor showed the highest biomass retention (sludge recovered), while the control retained the least

Table 3 | Characteristics of biomass inoculums used for reactors startup, and final sludge recovered after study

		Final sludge recovered after 21 days				
Parameter	**Inoculum seed (day 0)**	***Control***	***ZVNI***	***MTS***	***AMDS***	***LS***
Biomass (mg/L)	5,000	1,550	3,230	2,810	2,750	1,825
Mean granule size (in μm)	102 ± 60	129 ± 7	167 ± 101	191 ± 126	251 ± 185	130 ± 118
% of granule ≥150 μm (%)	2	13.9	33.8	42.2	53.4	18.1
Sugar in EPS (mg/g TSS)	115	66	98	95	99	72
Protein in EPS (mg/g TSS)	1.5	1.2	1.2	1.1	1.1	1.3

amount of biomass (Table 3). The order of biomass retention correlated well with the iron contents in the additives. Clustering of microorganism in solid phase also accelerated sludge granulation, thereby increasing granule sizes and ensuring better biomass retention (Tiwari *et al.* 2004). All the reactors, including control, showed granulation to various degrees (Figure 3).

The observed mean granule sizes, and percentage of particles ≥150 μm are listed in Table 3. The extent of granulation appeared to be the highest in AMDS, followed by MTS, ZVNI, LS and control, and correlated well the difference in surface charge between additives and biomass (Figure 3). This was in agreement with the earlier studies showing additives promoting granulation with granule size correlating with the surface charge difference between additive and biomass (Jia *et al.* 1996). The protein content in the EPS was observed to be very low compared to its sugar content in inoculum, as well as final sludge recovered from all reactors. This could be presumably due to reduction of the protein under anaerobic condition from where sludge was sourced. Moreover, EPS compositions in sludge depends on various other factors, and anaerobic granular sludge has shown low EPS-protein levels under various conditions in the past (Kobayashi *et al.* 2015).

Mechanism of biostimulation

The two mechanisms were postulated that presumably influence adhesion and joining of microorganisms to the additive material's surface. The differences in surface charge between the additive material and biomass leads to a partial positive charge on the surface of the additives which induced the phenomenon of adhesion through attractive forces (Figure 4). In addition, highly porous and

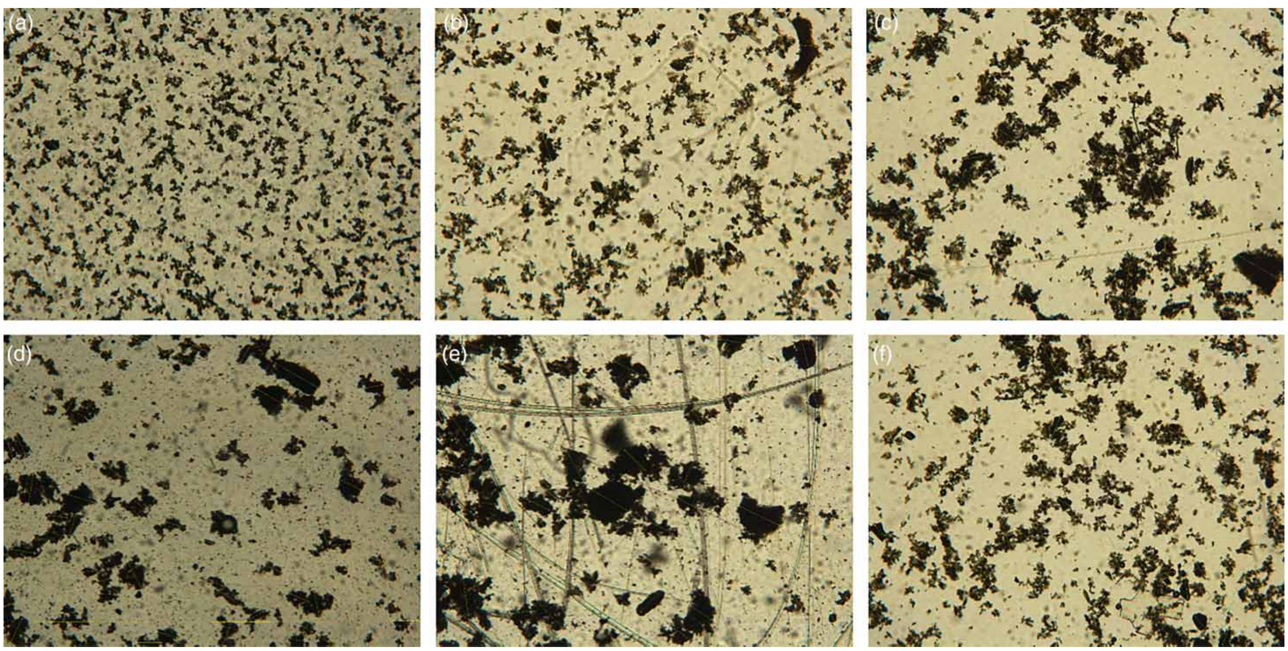

Figure 3 | Sludge aggregates in the process of forming granules (viewed under optical microscope at ×4 resolution): (a) inoculum, (b) control, (c) ZVNI, (d) MTS, (e) AMDS, (f) LS.

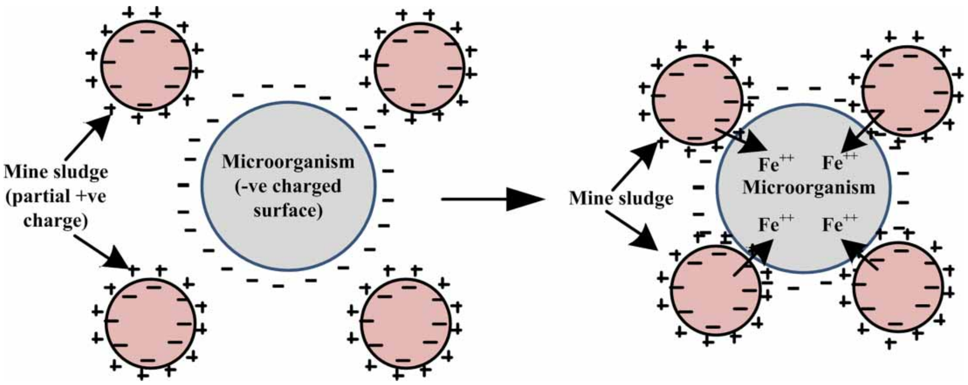

Figure 4 | Mechanism of adhesion of high –ve charge microorganism with less –ve charge mine sludge surface by inducing partial +ve charge on mine sludge surface and subsequent iron uptake by microbes from it.

irregular surface contributed by the presence of high iron content which helps in trapping microbial cell extension and fragments into its pores making cells to attach firmly to the additive's surface (Montanaro & Arciola 2000). Adhesion of the two resulted in formation of a biomass-additive composite which further turned into nucleation sites for adhesion of other additive particles and biomass resulting in formation of larger mass, i.e. sludge granule (Figure 4). Further, each granule acts as a separate anaerobic unit whose performance is dependent on the percentage of iron content in the granule (Wang *et al.* 2017). Inside the granules, microorganisms release metal solubilizing agents for taking up iron and other essential metals for promoting enhancement in metabolism, growth, EPS release and, subsequently, enhancement in removal efficiency of organics. High resolution SEM images showed various species of flourishing bacteria surrounded by MTS, appearing to extricate metals through it (Figure 5).

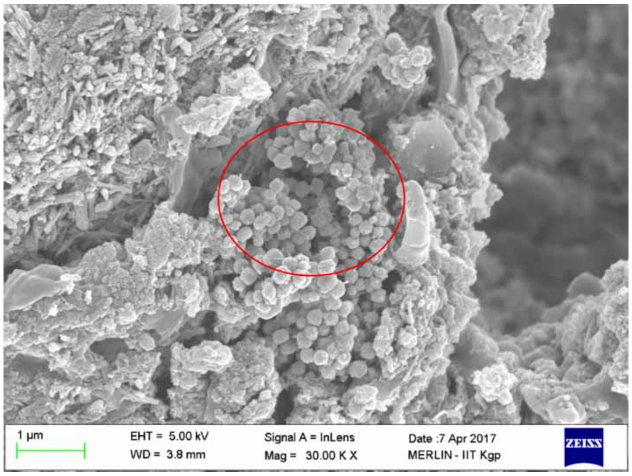

Figure 5 | Scanning electron microscope (SEM) image of colony of coccus bacteria attached with mine tailing sludge (MTS).

In the anaerobic microbial systems, high iron content is known to induce higher synthesis of EPS, which is a key substance for maintaining the integrity of a granule (Liu *et al.* 2004). EPS extracted from the sludge of all the reactors were observed to be correlated with the percentage of iron content in the additives. Therefore, it could be inferred that the difference in surface charge was keeping the microbes adhered to the additives, as a result, reducing VSS concentration in the effluent, whereas the high iron content is contributing to increase growth and metabolism of the microbes, thereby increasing the efficiency of COD removal.

Applicability and limitations of mine wastes in anaerobic treatment

The study demonstrated the potential of mine sludge wastes, MTS and AMDS, for improving performance of anaerobic wastewater treatment systems. Mixing of mine wastes together with anaerobic sludge from a septic tank or other sources could produce an effective inoculum for any anaerobic treatment process. The approach is better suited for the treatment of sewage generated at mine sites due to easy availability of mine waste in mining areas, else impacts related to transportation and off-site handling of mine waste will also have to be considered. The study was the first attempt of its kind, and further detailed investigations are recommended, including long term performance reliability and risk analysis, especially the potential of metal leaching from mine waste. Further, scale-up experiments and process optimization should be considered before implementation.

CONCLUSION

The iron rich mine waste supplements MTS and AMDS used as additives in anaerobic sewage treatment, resulted in reduced start-up time, higher steady state COD removal efficiency, lower VSS in effluent and higher methane recovery compared to control, as well as lower-iron containing additive LS. However, a synthetic iron-rich material ZVNI showed higher treatment efficiency than mine waste based additives. Difference in surface charge between additives materials and biomass was observed to influence adhesion of biomass with additives helping to develop larger granules. Although various metals were present in the mine waste, the efficiency of process was observed mainly correlated with the percentage of iron content. Higher iron content inside the granule promotes an increase in microbial metabolism and subsequently increases treatment efficiency of domestic sewage by enhancing acidogenesis and methanogenesis. The study not only proposed a solution for mine sludge reuse, but also integrated its reuse for managing another environmentally critical issues of wastewater treatment. However, further optimization and risk and reliability analysis is recommended to ensure the adaptability of the developed approach.

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