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Study of microbial contribution in alkalinity generation during treatment of acid mine drainage by laboratory successive alkalinity producing system

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Presently, the acid mine drainage (AMD) is a very common environmental problem being faced by mining communities throughout the world. The AMD generated in the mines is characterized by low pH value which results in further dissolution of minerals and release of toxic metals into the water. The successive alkalinity producing system (SAPS) is a passive treatment system which has evolved to harness the treatment benefits of limestone and wetlands together. In this laboratory study four identically designed SAPS were operated simultaneously with four different types of synthetic AMD for different Hydraulic Retention Time (HRT). The cow compost, sawdust and limestone were used in SAPS. The % contribution of organic substrate in net alkalinity generation due to microbial activities was measured for different HRTs. In this study is observed that organic substrate has contributed 70.96% and limestone layer contributed 29.04% in net alkalinity generation by SAPS.

Keywords: Acid mine drainage, Hydraulic retention time, Successive alkalinity producing system

The acid mine drainage (AMD) generated in the mines is characterized by low pH value which results in further dissolution of minerals and release of toxic metals into the water. The main purpose of AMD treatment systems is to lower acidity and toxic metal concentrations, raise pH and often lower sulfate concentrations and salinity. Mining of the coal and metals exposes the pyrite minerals to oxygen and water, which coupled with bacterial activity, leads to formation of AMD that are highly enriched with sulfate, aluminium and heavy metals[1-3].

Hedin, Watzlaf & Nairn (1994) indicated that the coal mine drainages in the U.S.A[4] are generally contaminated with dissolved iron, aluminium and manganese. Barnes & Romberger and Kleinmann *et al*. (as cited in Watzlaf, 2004) has stated the following chemical reactions, which occurs during formation of AMD[5,6].

$$FeS_2 + 3.5\ O_2 + H_2O \xrightarrow[\text{Bacteria}]{} Fe^{2+} + 2\ SO_4^{2-} + 2H^+ \quad \ldots (1)$$

$$Fe^{2+} + 2.5\ H_2O + 0.25\ O_2 \rightarrow Fe\ (OH)_3 + 2H^+ \quad \ldots (2)$$

$$Fe^{2+} + 0.25\ O_2 + H^+ \xrightarrow[Bacteria]{} Fe^{3+} + 0.5H_2O \quad \ldots (3)$$

$$FeS_2 + 14Fe^{3+} + 8H_2O \rightarrow 15Fe^{2+} + 2SO_4 + 16H^+ \quad \ldots (4)$$

In the first reaction sulfide mineral is oxidized to sulfate and acidity (H^+) is produced and Fe^{2+} gets dissolved in water. Then in reaction (2) Fe^{2+} reacts with water and oxygen and $Fe(OH)_3$ precipitates and again acidity H^+ is produced. In the reaction (3) some acidity (H^+) is consumed and further reaction begins and Fe^{3+} ion is formed and finally in reaction (4) Fe^{3+}, FeS_2 and water reacts and produce high acidity *i.e.* $16H^+$. Jage (2000) stated that the oxidation of FeS_2 become faster in presence of Fe^{3+} which generates 16 moles of acidity after reaction with each mole of FeS_2[7]. Zhang *et al*. (as cited in Sheoran, Sheoran, & Choudhary 2010) highlighted that the above reaction (4) shows the polluting capability of the oxidation of pyrite that every mole of pyrite can be converted to Fe^{2+} and regenerated to 16 mol of hydrogen and 2 mol of sulfate[8,9]. The iron-oxidizing bacteria and archaea such as *Acidithiobacillus ferrooxidans*, which oxidizes Fe^{2+} to Fe^{3+} and reduces sulfur species (Thiosulfate and sulfur), and *Leptospirillum ferrooxidans*, which oxidizes only Fe^{2+}, plays important role in AMD generation. Nordstrom (2011) indicated that in absence of Fe-oxidizing bacteria, pyrite oxidation stops since the abiotic oxidation of Fe^{2+} at low pH is much too slow[10]. Natarajan (2008) stated that the acidophilic autotrophic bacteria consume ferrous ion and sulfur compounds as their energy source and reproduce through binary fission

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up to 10^8 cells/mL in water[11]. Jacobs *et al*. (2014) described that the presence of microbial population not only plays important role in AMD generation, but it also increases the corrosion and bioleaching of metals[12]. In highly acidic AMD the ferric iron is predominately found. Ferric iron exists in insoluble form near neutral pH[13], whereas ferric iron is soluble in lower pH level. The major share about 75% of AMD generation is contributed by microbial activity[14,15].

Treatment of AMD

Kuyucak (1999) suggested that in dealing with AMD, one should focus on minimization of generation of AMD[16]. If generation of AMD cannot be prevented, it must be collected and treated**.** Broadly two types of AMD treatment methods are used worldwide *i.e*.

- Active treatment method and
- Passive treatment method

Skousen *et al*. cited in Jage (2000) described that in active treatment method mainly chemicals like sodium hydroxide, ammonia, hydrated lime, quick lime or soda ash *etc*. are used to raise the pH of water[7]. Clyde, Champagne, Jamieson & Gorman (2016) stated that passive treatment system is basically low energy environmentally sustainable AMD treatment system[17]. The concept of SAPS was first reported by Hendricks in 1991 and then it was modified by Kepler and Mccleary in 1994[18]. SAPS is a modified form of anaerobic wetlands provided with additional drainage pipe provided at the bottom of limestone layer with a flush valve and standpipe which help in maintaining sufficient head of water in SAPS column for downward movement of AMD solution. Anaerobic digestion produced methane which is not the case with SAPS[18,19]. SAPS have advantages of anaerobic wetlands and efficiency of anoxic limestone drain[20]. SAPS are also known as reducing and alkalinity producing system (RAPS) or vertical flow reactor (VFR) or vertical flow wetlands (VFW). Younger, Curtis, & Pennell (1997) stated that the topography is one of the key constraints for installation of SAPS because sufficient head should be available for causing vertical flow of AMD[21].

Materials and Methods

Sufficient quantity of synthetic AMD were prepared in the laboratory having variations in Iron, Aluminum and Manganese content for these experimental investigations. The parametric variations in preparation of synthetic AMD was carried out in a particular range to represent the composition of coal mine AMD reported in various literatures as well as by collecting some samples from coal mines. The research work comprised of performing experimental investigations on the four identical laboratory SAPS units, wherein four varieties of synthetic AMDs of a predetermined composition were treated. One additional unit of SAPS was also run for blank column test to see the variation in pH of influent AMD sample for 24 days. The SAPS treatment process was performed at 1-10 day retention times, with four SAPS units working together at a given time.

Samples were drawn from the influent end of the SAPS unit, at half depth of the organic layer, bottom of the organic layer, and at the discharge of the SAPS unit. Five retention times (1 day, 2 days, 4 days, 7 days and 10 days) were experimented for each AMD. Twenty samples were obtained during the treatment of each AMD. The samples were tested in laboratory as per the American Public Health Association (APHA) standard methods[22].

SAPS Experimental set-up

SAPS component

The SAPS system consists of three components (i) an influent AMD tank of 80 L capacity, which is kept at higher elevation to facilitate the flow of AMD, (ii) the SAPS unit which is filled with organic substrate and limestone having 80 L capacity as shown in (Fig. 1) and (iii) oxidation cell in which AMD from SAPS unit is discharged.

Design of SAPS column

Four 80 L PVC containers were taken for fabrication as SAPS units. In SAPS units bottom 1.25 cm diameter perforated PVC pipe is fitted for the purpose of flushing and discharge of processed AMD with the help of standpipe to oxidation cell. Then a limestone (size 1-2 cm) layer of 15 cm thickness is filled up after that saw dust and cow compost layer are filled up with 5 cm and 22 cm thickness, respectively. Then 2.5 cm thick gravel is packed at the top and finally AMD water is allowed to fill up to 15 cm height above the top gravel pack. The estimation of quantity of limestone and organic substrate were done as per chemical calculations. The estimated quantity of limestone is 27 kg (approx.) and volume of organic substrate was taken as 29 L (approx.). Limestone of following composition was collected

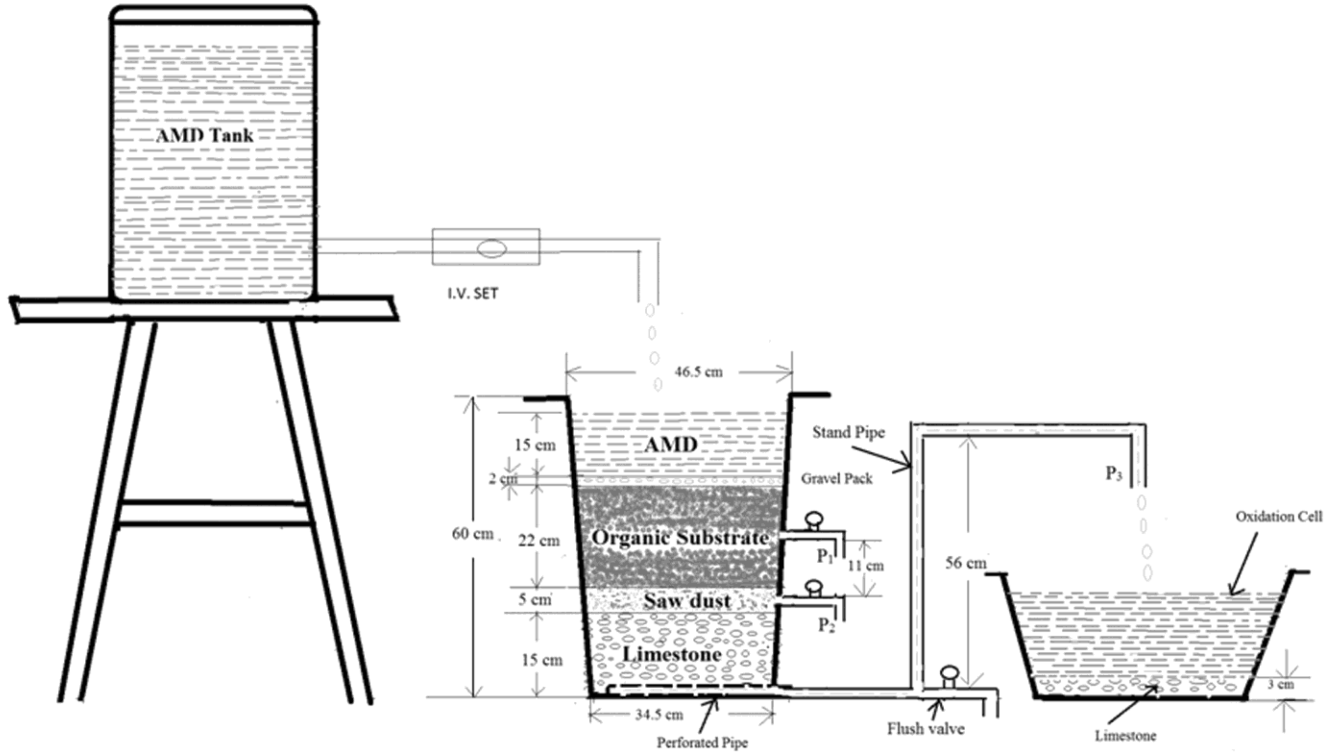

Fig. 1 — Laboratory arrangements for SAPS column study[23]

Table1 — Composition of limestone used in SAPS

S. No.	Element	Percentage
1	CaO	46.94
2	Fe_2O_3	0.64
3	SiO_2	7.90
4	Al_2O_3	2.04
5	MgO	0.79
6	K_2O	0.92

from Baikunth Limestone Mines of Century Cement Limited Chhattisgarh (India) given in (Table 1).

A polyethylene net of 16 mesh is placed between different layers of materials to avoid the mixing and for maintaining the easily flow condition inside the SAPS unit and the oxidation cell allows the oxic conditions for water coming from the SAPS unit, where metal gets precipitated and pH further increases. In each SAPS system, the oxidation cell of 50 L volume was attached having 3 cm thick layer of limestone at the bottom as presented in (Fig. 1). The method of filling limestone, cow compost, saw dust and synthetic AMD are shown in (Figs. 2A-C & 3), respectively. The cow compost is taken from agricultural farm having sufficient number of microbial counts about 2.96×10^5 cfu/mL. This microbial population is sufficient for AMD treatment in SAPS units.

Experimentation conditions

All the experiments were performed in identical conditions with the temperature ranges from 28.0-43.3°C. In this experiment four identical SAPS units having similar composition of limestone and organic substrate were operated simultaneously. The physiochemical characteristics analysis, elemental analysis and microbial analysis of cow compost were performed before the start of experiment. The physicochemical characteristics of cow compost were found similar. The results of above analysis are given in (Table 2).

Experimental methodology

In this study five hydraulic retention time (HRTs) of 1 day (1d), 2 days (2d), 4 days (4d), 7 days (7d) and 10 days (10d) were chosen. The flow rate was maintained with the help of intravenous infusion set (I-V set). The flow rate and corresponding HRT are shown in (Table 3).

Sampling process

Sampling was done at following four locations in the SAPS system.

1. Influent AMD (INF)
2. Midpoint of organic layer (P_1)
3. Interface of saw dust and limestone bed (P_2)
4. Effluent of SAPS unit (Standpipe) (P_3)

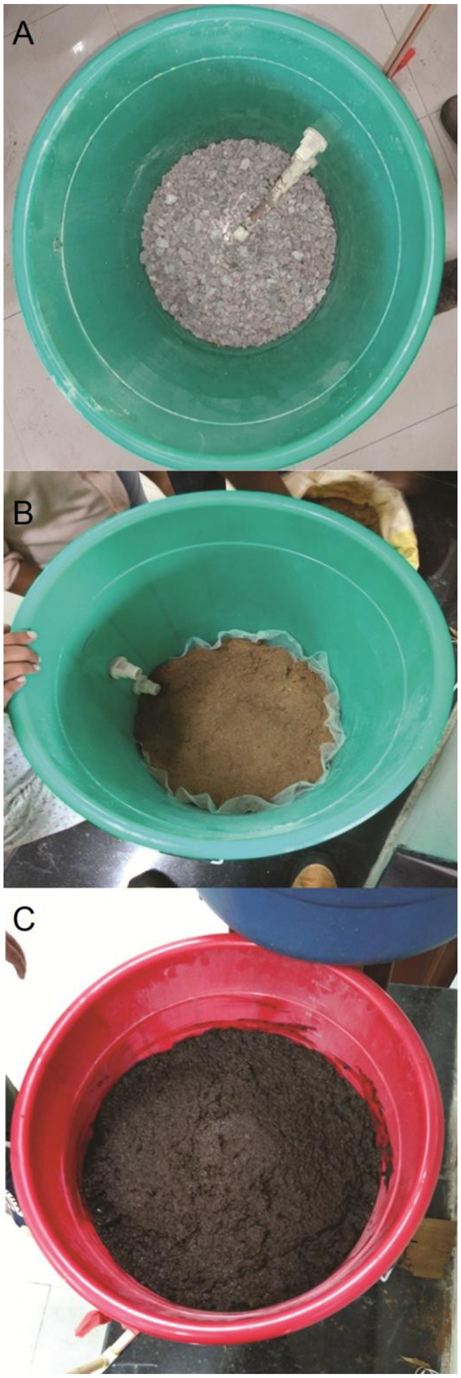

Fig. 2 — (A) Limestone layer filling in SAPS unit; (B) Saw dust layer filling in SAPS unit; and (C) Cow compost filling in SAPS unit

The net alkalinity generated by microbial population can measured at port P_2, however at port P_3 total alkalinity generation can be measured.

Fig. 3 — Filling of synthetic AMD in influent tank

Table 2 — Physicochemical characteristics of cow compost used in SAPS column

Parameters	Value
pH	6.68
Water content (%)	58.3
Nitrogen (%)	1.08
Carbon (%)	18.40
Hydrogen (%)	5.82
Sulfur (%)	< 0.01
C/N Ratio	17.03
Microbial count cfu/mL (colony forming unit /mL)	2.96×10^5

Table 3 — Hydraulic retention time and corresponding flow rate in SAPS

S. No.	Hydraulic Retention Time (HRTs) in days	Flow Rate mL/min
1	1d	28
2	2d	14
3	4d	7
4	7d	4
5	10d	2.8

Sample Testing

The American Public Health association (APHA) standards were followed during sample testing (Baird, Eaton, & Rice. 2017). All the samples for each SAPS units are taken for measurement and analysis at port P_1 and port P_2. The pH, DO, ORP, temperature and electrical conductivity are instantly measured by portable WTW multi 3620 IDS digital meter. Then collected samples were filtered in 0.45 micrometer Whatman membrane filter and nitrified with HNO_3 and kept for further analysis in refrigerator at 4°C. Then alkalinity was determined by 0.02N H_2SO_4titration and acidity was determined by 0.02N NaOH titration.

The determination of ferrous iron and total iron were carried out using 1, 10-phenantroline solution and ammonium acetate buffer solution by spectrophotometer. The ferric iron was calculated as difference of total iron and ferrous iron. Aluminum was determined using erichrome cyanine R spectrophotometer method. Manganese was determined using ammonium persulphate by spectrophotometer. Sulfate determination was done by using barium chloride method in spectrophotometer. The Lasany UV-VIS spectrophotometer was used in above mentioned tests. Calcium and magnesium were determined by EDTA method. Carbon, nitrogen, hydrogen and sulfur were determined by CNHS analyzer. The microbial counts were done by culturing on nutrient agar media as shown in (Figs. 4-6). The flow rate was measured by volumetric cylindrical flask and stop watch.

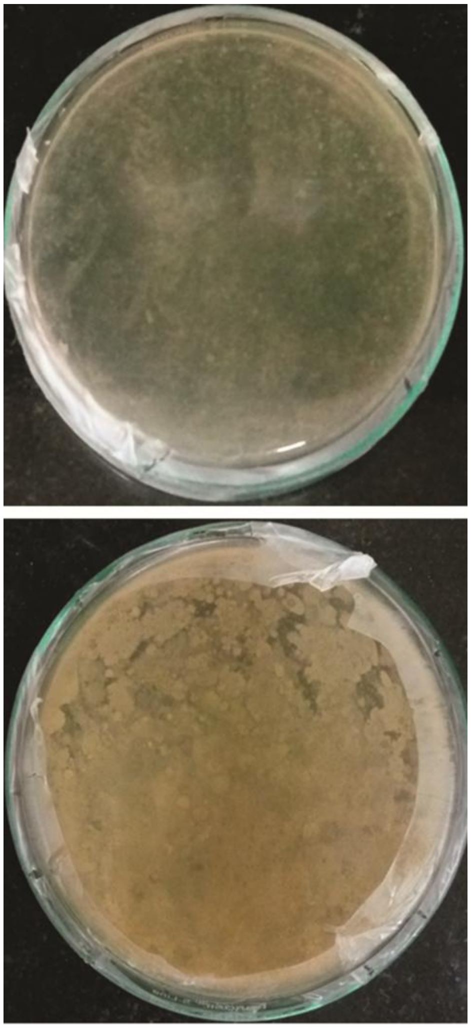

Fig. 4 — Spreading of water sample on the nutrient agar plate for the unit counting and identification of the bacterial colony

Observations and Results

In SAPS, the filled organic matter depletes the dissolved oxygen (DO) level due to its oxidation and degradation. A favorable anaerobic and reducing environment is attained after decreasing DO level. The microbial activity increases during acclimation period in anaerobic condition. The organic matter acts as electron donor and sulfate ions act as electron accepter. These sulfates are consumed by sulfate reducing bacteria (SRB) and H_2S gas is produced. The H_2S further reacts with dissolved metals and precipitated them in form of metal sulfide. Therefore, bicarbonate alkalinity is produced by organic matter. The above reduction reaction drops the ORP level from positive to negative zone.

Therefore, decrease in DO and ORP levels are indicators of favorable anaerobic and reducing environment inside the SAPS unit. Further, alkalinity

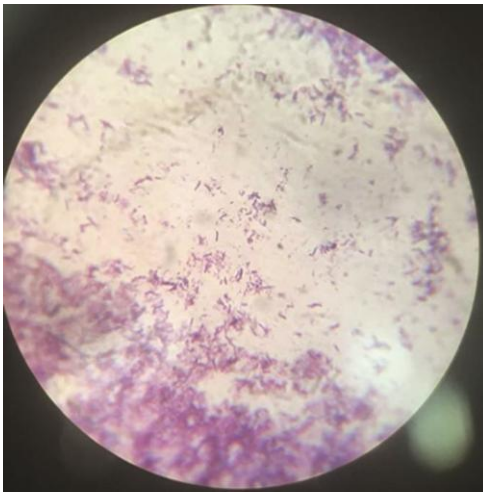

Fig. 5 — Unit counting of the bacteria

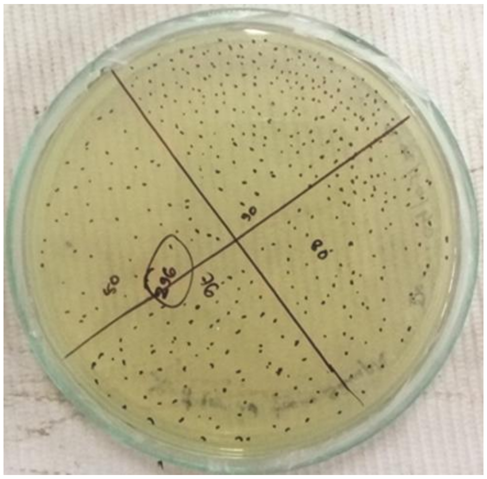

Fig. 6 — Gram staining of the bacteria, resulted Rod shaped gram-positive bacteria

is generated by dissolution of limestone at bottom of the SAPS unit.To ascertain the performance of SAPS the AMDs were processed for above parameters after 15 days acclimation period and attainment of perfect anaerobic condition, which is indicated by strong smell of H_2S and visible black film layer of SRB. The performance of SAPS is evaluated in terms of net alkalinity generated (NAG). In this research work the net alkalinity generated (NAG) is taken as sum of acidity reduced and alkalinity increased. The NAG is expressed in terms of $CaCO_3$ equivalence in mg/L.

Net alkalinity generation at Port P_1

The net alkalinity generation at Port $P_1$for AMD A_1 was observed to be 155 mg/L, 210 mg/L, 275 mg/L, 410 mg/L and 465 mg/L for 1d, 2d, 4d, 7d, and 10d HRTs, respectively, with the corresponding pH level of 5.40, 6.00, 6.10, 6.80 and 6.90, respectively, as shown in (Fig. 7A). The net alkalinity generation increased with increased retention time. Therefore, alkalinity generation is increases with increase in HRT duration because more time were available for microbial reactions.

The net alkalinity generation at Port P_1 for AMD B_1 was observed to be 205 mg/L, 215 mg/L, 300 mg/L, 450 mg/L and 525 mg/L for 1d, 2d, 4d, 7d, and 10d HRTs, respectively, with the corresponding pH level of 5.90, 6.00, 6.30, 6.70 and 6.80, respectively, as shown in (Fig. 7A). Therefore, net alkalinity generation increased with increased retention time.

The net alkalinity generation at Port P_1 for AMD C_1 was observed to be 275 mg/L, 325 mg/L, 405 mg/L, 590 mg/L and 660 mg/L for 1d, 2d, 4d, 7d, and 10d HRTs, respectively, with the corresponding pH level of 5.60, 5.80, 5.90, 6.60 and 6.70, respectively, as shown in (Fig. 7A). Therefore, net alkalinity generation increased with increased retention time.

The net alkalinity generation at Port P_1 for AMD D_1 was observed to be 380 mg/L, 390 mg/L, 435 mg/L, 695 mg/L and 755 mg/L for 1d, 2d, 4d, 7d, and 10d HRTs, respectively, with the corresponding pH level of 5.10, 5.60, 5.70, 6.70 and 6.80, respectively, as shown in (Fig. 7A). Therefore, net alkalinity generation increased with increased retention time. (Table 4)

Net alkalinity generation at Port P_2

The net alkalinity generation in SAPS unit was further increased at Port $P_2$for AMD A_1. The observed alkalinity generation at Port $P_2$for AMD A_1 was 280 mg/L, 325 mg/L, 485 mg/L, 620 mg/L and 765 mg/L for 1d, 2d, 4d, 7d, and 10d HRTs, respectively, with the corresponding pH level of 5.90, 6.10, 6.40, 7.10 and 7.40, respectively, as shown in (Fig. 7B). In this

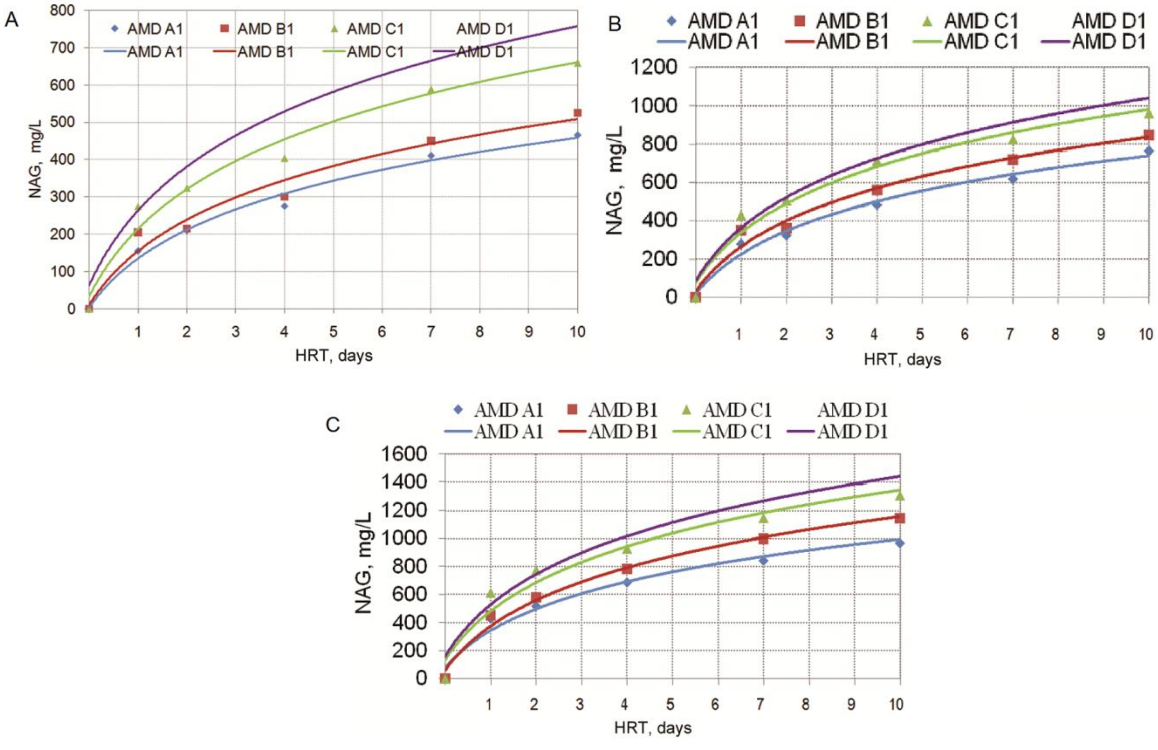

Fig. 7 — NAG in different HRT for Port (A) P_1; (B) P_2; and (C) P_3

zone the alkalinity is generated by microbial activity in organic substrate layer.

The net alkalinity generation in SAPS unit was further increased at Port P_2 for AMD B_1. The observed alkalinity generation at Port P_2 for AMD B_1 was 350 mg/L, 360 mg/L, 560 mg/L, 720 mg/L and 845 mg/L for 1d, 2d, 4d, 7d, and 10d HRTs, respectively, with the corresponding pH level of 6.10, 6.30, 6.80, 7.00 and 7.20, respectively, as shown in (Fig. 7B).

The net alkalinity generation in SAPS unit was further increased at Port P_2 for AMD C_1. The observed alkalinity generation at Port P_2 for AMD C_1 was 430 mg/L, 510 mg/L, 715 mg/L, 825 mg/L and 960 mg/L for 1d, 2d, 4d, 7d, and 10d HRTs, respectively, with the corresponding pH level of 6.50, 6.60, 6.60, 6.80 and 7.00, respectively, as shown in (Fig. 7B).

The net alkalinity generation in SAPS unit was further increased at Port P_2 for AMD D_1. The observed alkalinity generation at Port P_2 for AMD D_1 was 510 mg/L, 515 mg/L, 710 mg/L, 895 mg/L and 1030 mg/L for 1d, 2d, 4d, 7d, and 10d HRTs, respectively, with the corresponding pH level of 5.90, 6.20, 6.40, 6.80 and 6.90, respectively, as shown in (Fig. 7B).

Net alkalinity generation at Port P_3

The generation of net alkalinity increases at Port P_3 because of dissolution of limestone in the bottom layer. The net alkalinity generation at Port P_3 for AMD $A_1$was observed to be 430 mg/L, 520 mg/L, 690 mg/L, 845 mg/L and 970 mg/L for 1d, 2d, 4d, 7d, and 10d HRTs, respectively, with corresponding pH level of 6.40, 6.40, 6.80, 8.50 and 8.60, respectively, as shown in (Fig. 7C). The maximum net alkalinity generated is observed to be 970 mg/L for 10d HRT.

The net alkalinity generation at Port P_3 for AMD $B_1$was observed to be 450 mg/L, 580 mg/L, 785 mg/L, 995 mg/L and 1140 mg/L for 1d, 2d, 4d, 7d, and 10d HRTs, respectively, with corresponding pH level of 6.20, 6.70, 7.00, 8.20 and 8.60, respectively, as shown in (Fig. 7C). The maximum net alkalinity generated is observed to be 1140 mg/L for 10d HRT.

The net alkalinity generation at Port P_3 for AMD $C_1$was observed to be 615 mg/L, 775 mg/L, 930 mg/L, 1145 mg/L and 1305 mg/L for 1d, 2d, 4d, 7d, and 10d HRTs, respectively, with corresponding pH level of 6.70, 6.80, 6.90, 8.20 and 8.50, respectively, as shown in (Fig. 7C). The maximum net alkalinity generated is observed to be 1305 mg/L for 10d HRT.

The net alkalinity generation at Port P_3 for AMD $D_1$was observed to be 690 mg/L, 830 mg/L, 1010 mg/L, 1220 mg/L and 1400 mg/L for 1d, 2d, 4d, 7d, and 10d HRTs, respectively, with corresponding pH level of 6.10, 6.90, 7.00, 8.30 and 8.40, respectively, as shown in (Fig. 7C). The maximum net alkalinity generated is observed to be 1400 mg/L for 10d HRT.

Table 4 — Composition of AMD used in experiment

Parameters	AMD A_1	AMD B_1	AMD C_1	AMD D_1
pH	4.50	3.70	2.80	2.60
ORP (mV)	107.50	109.70	111.40	113.50
DO (mg/L)	7.13	6.89	6.78	6.97
Total Fe (mg/L)	85.7	118.7	171.6	195.5
Al (mg/L)	20	20	20	20
Mn (mg/L)	15	15	15	15
Ca (mg/L)	125	125	125	125
Mg (mg/L)	100	100	100	100
SO_4^{2-}(mg/L)	1020	1028	1030	1026
Electrical conductivity µs/cm	1939	1985	2140	2160

Contribution of organic matter in alkalinity generation

The contribution of organic matter in alkalinity generation is substantially higher as compared to limestone. The share of microbial activities in alkalinity generation was found 71.68%, 63.11%, 72.20%, 72.78%, and 75.02% for 1d, 2d, 4d, 7d and 10d HRT, respectively, as shown in (Tables 5-9). Therefore it is obvious that organic matter play important role in alkalinity generation in SAPS.

The overall average of net alkalinity generation contribution by organic layer is found 70.96% and 29.04% alkalinity generation is contributed by limestone layer for all HRTs.

Table 5 — Share of NAG by organic layer and limestone layer for HRT= 1d

AMD	Alkalinity at port P_2	Alkalinity at port P_3	Difference in Alkalinity (P_3-P_2)	% Alkalinity generation due to organic layer P_2/P_3	% Alkalinity generation due to lime stone layer (P_3-P_2)/P_3
A_1	280	430	150	65.12	34.88
B_1	350	450	100	77.78	22.22
C_1	430	615	185	69.92	30.08
D_1	510	690	180	73.90	26.10
				Av.=71.68	Av.=28.32

Table 6 — Share of NAG by organic layer and limestone layer for HRT= 2d

AMD	Alkalinity at port P_2	Alkalinity at port P_3	Difference in Alkalinity (P_3-P_2)	% Alkalinity generation due to organic layer P_2/P_3	% Alkalinity generation due to lime stone layer (P_3-P_2)/P_3
A_1	325	520	195	62.50	37.50
B_1	360	580	220	62.07	37.93
C_1	510	775	265	65.80	34.20
D_1	515	830	315	62.05	37.95
				Av.=63.11	Av.=36.89

Table 7 — Share of alkalinity generation by organic layer and limestone layer for HRT= 4d

AMD	Alkalinity at port P_2	Alkalinity at port P_3	Difference in Alkalinity (P_3-P_2)	% Alkalinity generation due to organic layer P_2/P_3	% Alkalinity generation due to limestone stone layer (P_3-P_2)/P_3
A_1	485	690	205	70.29	29.71
B_1	560	785	225	71.34	28.66
C_1	715	930	215	76.88	23.12
D_1	710	1010	300	70.29	29.71
				Av.=72.2	Av.=27.8

Table 8 — Share of NAG by organic layer and limestone layer for HRT= 7d

AMD	Alkalinity at port P_2	Alkalinity at port P_3	Difference in Alkalinity (P_3-P_2)	% Alkalinity generation due to organic layer P_2/P_3	% Alkalinity generation due to lime stone layer (P_3-P_2)/P_3
A_1	620	845	225	73.37	26.63
B_1	720	995	275	72.36	27.64
C_1	825	1145	320	72.05	27.95
D_1	895	1220	325	73.36	26.64
				Av.=72.78	Av.=27.22

Table 9 — Share of NAG by organic layer and limestone layer for HRT= 10d

AMD	Alkalinity at port P_2	Alkalinity at port P_3	Difference in Alkalinity (P_3-P_2)	% Alkalinity generation due to organic layer P_2/P_3	% Alkalinity generation due to lime stone layer (P_3-P_2)/P_3
A_1	765	970	205	78.86	21.14
B_1	845	1140	295	74.12	25.88
C_1	960	1305	345	73.56	26.44
D_1	1030	1400	370	73.57	26.43
				Av.=75.02	Av.=24.98

Discussion

The increase in NAG from 1d to 10d HRT at Port P_1 for AMD A_1 was (465-155)=310 mg/L whereas the cumulative difference up to Port P_2 for 1d HRT to 10d HRT was (765-280)= 485 mg/L. It was found that NAG is dependent on HRT. The above findings showed that rate of NAG was initially faster then became slower. Similar findings were also found for AMD B_1, AMD C_1, and AMD D_1. It also reveals that rate of NAG got slower due less availability of carbon due consumption of carbon present cow compost for longer HRT.

The microbial contribution percentage in NAG was assessed at Port P_2, which was 65.12% for AMD A_1, 77.78% for AMD B_1, 69.92% for AMD C_1 and 73.90% for AMD D_1 for 1d HRT. Similar findings were also found for 2d, 4d, 7d and 10d HRT. Therefore, it was concluded that the average share of microbial contribution in NAG were ranged from 63.11% to 75.02% in the experiment for 1d HRT to 10 HRT and not dependent on HRT. Thus, the microbial contribution is much higher than the limestone contribution. Therefore, the average contribution due to microbial activities in SAPS was found to be 70.96%.

The limestone contribution percentage in NAG was assessed at Port P_3 by subtracting NAG at Port P_3 and

Port P_2. The NAG share by limestone was ranged from 24.98% to 36.89% for different HRT. Therefore, it was concluded that contribution of limestone in NAG is not dependent on HRT.

Conclusion

An investigation had been carried out to study of microbial contribution in alkalinity generation through laboratory Successive Alkalinity Producing System for AMD treatment. Based on the experimental studies, some key findings have been obtained like (a) Cow compost was found effective in net alkalinity generation by microbial population during treatment of AMD by SAPS; (b) the net alkalinity generation by microbial is found increasing with increase in HRT; (c) the trend of NAG increase found in logarithmic nature; (d) the share of microbial NAG were 71.68%, 63.11%, 72.2%, 72.78% and 75.02% of total NAG for 1d, 2d, 4d, 7d and 10d, respectively; (e) the overall average of net alkalinity generation contribution by organic layer is found 70.96%, for all HRTs and remaining 29.04% alkalinity generation contributed by limestone layer.

Conflict of interest

All authors declare no conflict of interest.

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