



## Role of calcium oxide in sludge granulation and methanogenesis for the treatment of palm oil mill effluent using UASB reactor

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### ABSTRACT

The granulation process in palm oil mill effluent using calcium oxide–cement kiln dust (CaO–CKD) provides an attractive and cost effective treatment option. In this study the efficiency of CaO–CKD at doses of 1.5–20 g/l was tested in batch experiments and found that 10 g of CaO/l caused the greatest degradation of VFA, butyrate and acetate. An upflow anaerobic sludge blanket (UASB) reactor was operated continuously at 35 °C for 150 days to investigate the effect of CaO–CKD on sludge granulation and methanogenesis during start-up. The treatment of POME emphasized the influence of varying organic loading rates (OLR). Up to 94.9% of COD was removed when the reactor was fed with the 15.5–65.5 g-COD/g/l at an OLR of 4.5–12.5 kg-COD/m<sup>3</sup> d, suggesting the feasibility of using CaO in an UASB process to treat POME. The ratio of volatile solids/total solids (VS/TS) and volatile fatty acids in the anaerobic sludge in the UASB reactor decreased significantly after long-term operation due to the precipitation of calcium carbonate in the granules. Granulation and methanogenesis decreased with an increase in the influent CaO–CKD concentration.

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### 1. Introduction

Palm oil mill effluent (POME) is a colloidal suspension of 95–96% water and 4–5% total solids, including 2–4% suspended solids [1], with a biochemical oxygen demand (BOD) and chemical oxygen demand (COD) of 26.3 and 65.5 g/l, respectively. POME usually has a pH of 4–5 and contains appreciable amounts of nutrients for granulation [2]. The pH of POME is one of the key factors that influence its anaerobic digestion since methane producing bacteria require a neutral to slightly alkaline environment for granulation and methanogenesis. The optimum pH for granulation is between 6.8 and 7.2, while the pH values lower than 4 or higher than 9.5 are not tolerable [3,4]. Great attention has been paid to accelerate the start-up period and enhance anaerobic granule formation in UASB reactors with the aid of organic and inorganic chemical agents [5,6]. Minerals such as CaCO<sub>3</sub>, CaOH(PO<sub>4</sub>)<sub>3</sub>, AlSO<sub>4</sub>, and FeS are formed as a result of metabolic activities and physicochemical

reactions and are accumulated either inside or on the surface of granules [7,8]. Some metal ions, such as Ca<sup>2+</sup>, Fe<sup>2+</sup>, Al<sup>3+</sup>, Mg<sup>2+</sup>, CaO, and Ca(OH)<sub>2</sub>, enhance granulation and play an important role in microbial aggregation [9,10].

Anaerobic treatment of wastewaters including POME by UASB process is the most promising and useful technology [11–13]. The performance of UASB reactors is strongly dependent on anaerobic granular sludge [14]. The processes involved perform well at high organic loading rates (OLR) with low operating costs and also produce usable biogas [15]. Various anaerobic processes have been studied in recent years for the treatment of municipal wastes and landfill leachate, such as anaerobic digesters [16], fluidized bed reactors [17], sequencing batch reactors [18], up-flow anaerobic sludge blanket reactors [12,13,19], and anaerobic membrane bioreactors [20,21]. Of these reactor processes, UASB is the most promising technology since it works on a different scale and can be applied to different wastewater treatments. The granulation process is sensitive to sudden changes in environmental and operational fermentation conditions [22]. A great deal of research has been conducted on the functioning of a UASB reactor [23], its applicability to sewage treatment [24], treatment of some toxic wastes [25], nutrient requirements for the UASB process [26], and the mechanism of granulation [27,28]. The granules lose their strength and stability when decay starts at their centers due to substrate limitation [29]. However, granule disintegration is prevented when a UASB reactor is operated under low OLR (<1.5 kg-COD m<sup>3</sup>/day) and the highest biogas and methane production rates

*Abbreviations:* POME, palm oil mill effluent; VSS, volatile suspended solids; BOD, biochemical oxygen demand; MLSS, mixed liquor suspended solids; COD, chemical oxygen demand; MLVSS, mixed liquor volatile suspended solids; VFA, volatile fatty acid; HRT, hydraulic retention time; CaO–CKD, calcium oxide–cement kiln dust; GSL, gas–solid–liquid; UASB, up-flow anaerobic sludge blanket; SVI, sludge volume index; OLR, organic loading rate; BOD, biochemical oxygen demand; VS, volatile solids; EPS, extracellular polymeric substances; TS, total solids; CH<sub>4</sub>, methane.

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**Table 1**  
Characteristics of CKD.

Parameter	Dry-kiln CKD (% by weight)	Dry-kiln CKD (% by weight) <sup>a</sup>
pH	13.6 <sup>b</sup>	–
CaO	65	44.9
SiO <sub>2</sub>	11.6	9.64
Al <sub>2</sub> O <sub>3</sub>	6.1	3.39
Fe <sub>2</sub> O <sub>3</sub>	3.3	1.10
MgO	1.1	1.29
K <sub>2</sub> O	1.69	2.4
SO <sub>3</sub>	5.4	6.74
Particle size	<25 μm	1–40

<sup>a</sup> Adaska and Taubert [32].<sup>b</sup> Unitless parameter.

that have been achieved were 2.42 m<sup>3</sup>/m<sup>3</sup> and 0.992 m<sup>3</sup>/m<sup>3</sup> of reactor/day, respectively, at an OLR of 6.0 kg-COD/m<sup>3</sup>/day [30,31]. Therefore, dosing of CaO has been used as a method to accelerate the anaerobic granulation process.

This study was thus conducted to examine the treatability of POME and effects of CaO on the granulation process in UASB reactors. The main objective was to determine the influent CaO–CKD concentration and the relationship between the CaO concentration in the feed and biomass accumulation, specific granulation, methanogenic activity, density and composition of granules. The biomass concentration profiles along the reactors and the size distribution of granules were also measured to track and to assess the granulation, methanogenesis, and COD removal on levels at the industrial scale.

## 2. Materials and methods

### 2.1. Sample collection

A dry-kiln cement kiln dust (CKD) sample was collected from YTL Pahang Cement located in Kuantan city, Malaysia. The CKD sample was washed with water and filtered to obtain approximate particle sizes. Table 1 summarizes the characterization of the CKD sample used for POME treatment. The raw POME samples were collected from LHSC-Palm Oil Mill, situated in the state Pahang, Malaysia. The features and composition of the POME used are summarized in Table 2, which shows the average values of five replicate analyses for each parameter. These samples were stored at 4 °C. Various dilutions of POME were made with tap water. Each sample was pre-filtered by simple depth filtration to remove the coarse solids found in the suspension. The raw POME was initially passed through a filter bed, which consisted of minor stones with average size of 0.6 cm.

### 2.2. Slacking and calcium oxide

Lime slacking was conducted with different ratios of CKD in accordance with the method as described in the literature [32]. Bench scale experiments were carried out with five 500 ml flasks of POME to examine the neutralizing effects of CKD. Five different ratios of calcium hydroxide were used to examine the neutralizing effects after CKD slacking. The slacked solution of 10 g/l CaO–CKD was found to be the most suitable dose for the highest degradation of POME by significant granulation and methanogenesis at pH 6.5–7.5.

Calcium oxide in cement kiln dust extract solution was precipitated as calcium oxalate by adding ammonium oxalate. This addition made the medium slightly alkaline (because of ammonia). The calcium oxalate (white precipitate) was filtered and dissolved in dilute sulfuric acid. After the removal of oxalate and chloride ions

**Table 2**  
POME characteristics.

Parameter	General POME characteristics	EIA standards
Temp. <sup>a</sup>	55.5	45
pH	4.5	5.0–9.0
BOD	40,000	50
COD	65,000	100
sCOD	30,500	
VSS	20,000	400
TS	45,000	
TVS	26,300	
TP	950	
TOC	25,000	
O and G	1500	50
NH <sub>3</sub> -N	90	150
TKN	890	
TN <sup>b</sup>	945	200
VFA	1900	
SO <sub>4</sub>	5	
Lignin	130	
Zn	0.002	
Br	0.004	
Fe	0.005	
Mn	0.001	

All parameters are in mg/l except pH.

<sup>a</sup> °C.<sup>b</sup> As total nitrogen.

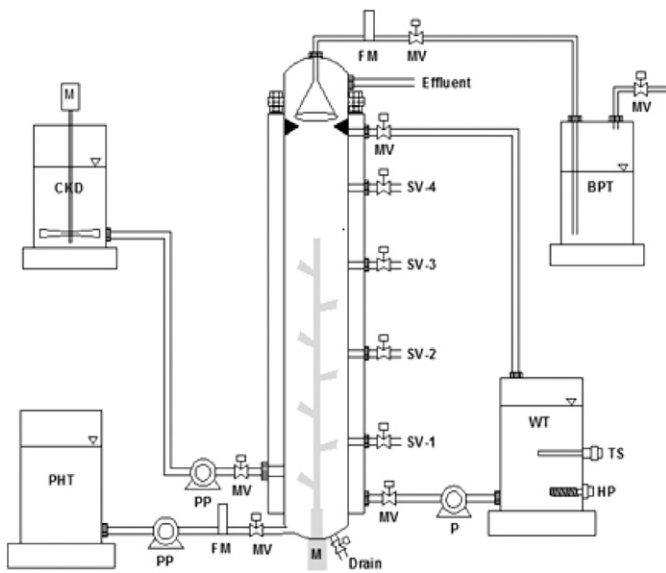
by washing, the liberated oxalic acid was titrated with a standard KMnO<sub>4</sub> solution in acidic medium at an elevated temperature. The amount of calcium oxide in cement extract could be determined from the volume of KMnO<sub>4</sub> solution used.

### 2.3. Batch experiments

Batch experiments were performed at both the natural pH of POME and at pH 7.5 (obtained by CaO addition) in a laboratory semi-batch column reactor consisting of a cylindrical tube 100 cm in height with an internal diameter of 4 cm. A Schott ceramic porous diffuser (porosity 4, 10–16 mm) was placed at the bottom of the reactor for CaO distribution. Methanogenic sludge was withdrawn from an anaerobic digester that had operated at an organic loading rate of 15 g-COD/l d at 35 °C for over 3 months. The nutrient medium consisted of 0.5 g/l NH<sub>4</sub>Cl, 15 g/l K<sub>2</sub>HPO<sub>4</sub>, 0.2 g/l MgCl<sub>2</sub>·6H<sub>2</sub>O, 0.05 g/l CaCl<sub>2</sub>, 1.5 g/l NaHCO<sub>3</sub>, and 1 ml/l trace element solution. Anaerobic conditions during the filling operation were maintained by continuous flushing with nitrogen gas. The semi-batch column reactor were submerged in thermostatic water baths (35 °C) and magnetically stirred. Samples were collected at regular intervals to analyze VFA, VSS, MLSS, COD, alkalinity, butyric, and acetate content.

### 2.4. Reactor operation

The experiments were performed in parallel in six identical 5 l UASB reactors continuously operated at 35 °C (Fig. 1). Heating of the reactor was done from hot water circulating through the water jacket. The feed was introduced from the bottom of the reactor by using peristaltic pump at a flow rate of 1.25 l/d and fixed hydraulic retention time (HRT) of 4 days. A gas–solid–liquid (GSL) separator was installed at top of the reactor for biogas capturing. Four sampling ports were installed at suitable heights of the reactor. Intermittent mixing was applied due to the lower volume and to avoid CaO–CKD from settling in the reactor. The experimental setup of the UASB reactor is shown in Fig. 1. In addition, the reactor containing granular sludge without added CaO was denoted as control (R1) while the reactors with added CaO concentration of 10, 12, 15, 18 and 20 g/l were designated as R2, R3, R4, R5 and R6. The UASB reactors were inoculated with these different doses of CaO–CKD



**Fig. 1.** Experimental setup of UASBR: PHT-POME holding tank; PP: peristaltic pump; FM: flow meter; MV: manual valve; M: mixture; SV: sampling valve; CKD: cement kiln dust tank (slaking solution); BPT: biogas purification tank; WT: water tank; TS: temperature sensor; HP: heating probe; P: pump.

and 350 ml of seed sludge. The sludge was acclimatized with POME daily by bench feeding diluted sludge (15 g-COD/L) for five days. The average volatile suspended solids (VSS) of the sludge after 5 days of being bench fed were 11.3 g/l. Continuous feeding was started with an initial organic loading rate (OLR) of 15 kg-COD/m<sup>3</sup> d and HRT of 4 days. The HRT was kept constant throughout the study. The influent COD concentration was 6 g/l for the first 20 days, and then it was increased stepwise. The reactor was monitored daily for volatile fatty acids, effluent VSS, MLVSS, and methane yields.

### 2.5. Analytical methods

Alkalinity was measured by the direct titration method [33]. Gas volume was measured by displacement of acidified water (pH 2–3) and methane by KOH solution displacement in a serum bottle, as described previously [4]. The operational parameters measured included COD removal efficiency, biogas production, biogas composition, effluent VSS concentration, pH, and particle size distribution. Sludge was sampled periodically from the sampling points arranged along the height of the reactors to determine the mixed liquor volatile suspended solids (MLVSS) concentration. Analysis of COD, dry weight and density of the biomass, VSS, MLVSS, SVI and BOD by (Winkler's methods) were performed according to standard methods [34].

The particle size distribution of the different CKD samples was determined by using a particle size analyzer (Model 2000E, Malvern). A major oxide analysis of each unhydrated CKD sample was done using ICP-OES (Vista-PRO Radial, Varian). Samples were

**Table 3**

Chemical analysis parameters of POME effluent treated at various CaO-CKD g/l dosages during fermentation time of 150 days in fermentation reactor.

Parameters	0 CaO	1.5	3.0	5	10	15	20
VFA	1930	1850	1720	1539	1080	1150	1256
VSS	3078	3173	3230	3363	3490	2550	2187
COD	5067	4090	4050	3010	2020	2180	3012
Alkalinity	3293	2789	2950	3535	4036	4862	5020
Butyrate	175	155	127	106	95	56	90
Acetate	369	495	560	620	610	650	715

\* All parameters are in mg/l.

first set by lithium metaborate/tetraborate fusion. The rapid sugar method was used to determine the available lime content as outlined in ASTM standard C 25-06. The suspensions were centrifuged at 10,000 rpm and supernatants were tested for VFA concentration. Headspace gas samples were collected regularly and analyzed for CH<sub>4</sub> gas. All tests were conducted in duplicate at 37 °C in a shaker at 150 rpm. VFAs were determined by gas chromatography with a FID detector (GC 8500 Perkin Elmer, glass 2 m × 2 mm packed column Carboxpack B-DA 80/120% CW 20 M, N<sub>2</sub> carrier at 220 kPa, 175 °C).

## 3. Results and discussion

### 3.1. Batch test experiment

The batch test performance of the different doses of CaO-CKD, concentrations of COD, VSS, and VFA in the influent, and the COD removal efficiencies are shown in Table 3. The batch test using CaO-CKD for the degradation of POME sludge depends mainly on the formation of stable micro-environment. The batch test with 10 mg/l CaO-CKD indicated healthy microbial activity by continuous increase in alkalinity (3293–4036) along with the maximum reduction (5067 g-COD/l to 2020 g-COD/l) of initial COD concentration. Interestingly, alkalinity increased with decreasing VFA and COD (Table 3). VFA degraded since more VSS and MLVSS were developed. The VSS and MLSS concentrations during the batch tests and biomass build-up phases are shown in Tables 3 and 4, respectively. During the acclimatizing phase, the SVI and MLVSS steadily rose to the values of 209 ml/g and 55,000 ml/l respectively. POME contains about 2300 mg/l acetate, and high suspended solids were more effective for enhancing methanogenesis and sludge granulation. The CaO-CKD doses varied between 1.5 and 20 g-CaO/l of CaO, while the granulation process lasted up to 150 days. The CaO-CKD efficiency of granulation was strongly dependent upon the CaO-CKD dose. In the present study, 10 g/l CaO-CKD was found to be most effective for maximum methanogenesis, for granulation to degrade organics, and for removal of COD. Higher doses of CaO-CKD did not significantly affect the granulation and methanogenic activity of the effluent [35]. COD removal was dependent on a CaO-CKD dose exceeding 75%. In particular, CaO-CKD at 10 g-CaO/l resulted in a COD removal of about 78% for the treated effluent.

**Table 4**

Effect of CaO-CKD in g/l on various parameters in POME at different fermentation batch reactor using butyrate as carbon source for the biomass.

CaO-CKD	MLSS	MLVSS	MLVSS/MLSS ratio	Influent COD	Sludge volume index (ml/g)	%COD removal
1.5	30,000	20,000	2200	45,000	35	39.5
3.0	3500	30,000	4300	45,000	120	61.9
5.0	37,000	35,000	3500	45,000	146	73.7
10.0	55,000	40,000	3100	45,000	209	78.6
15.0	41,000	35,000	2600	45,000	124	70.3
20.0	35,000	31,000	2100	45,000	112	62.5

\* All parameters are in mg/l.

### 3.2. COD removal

The performance of the reactors in terms of COD removal efficiency, effluent VSS concentration, granulation and methane production continuously improved with operation time (days). The operational conditions and performance of the reactor were measured for 150 days. At initial 20 days, the COD removal efficiencies were slow. The control reactor (R1) showed the highest COD in the effluent. On the other hand the reactors dosed with CaO (R2–R6) showed higher removal efficiencies. Thus, reactors infused with calcium oxide showed faster COD removal efficiencies rather than R1 (Fig. 2). When the OLR was increased from 6.5 to 12.5 g-COD/l d, the COD removal and performance of the reactor R1 was still lower than that of R2–R6. However, at an OLR of 12.5 g-COD/l d, R2 and R3 had almost the same level of COD removal efficiencies, while it was lower in the reactors R4–R6. The COD removal efficiency of R2 increased up to 97.8% after 95 days of operation which was comparatively higher than other reactors (R1, R3–R6). Further stepwise increase in OLR from 35.5 to 55.0 g-COD/l by increasing the influent COD concentration resulted continuous decrease in COD removal efficiency. Thus, the results showed that the R2 exhibited maximum COD removal efficiency of 97.8% at an OLR of 35.0 g-COD/l d and influent COD concentration of 45,000 mg/l. The removal of COD is reflected in the increasing biomass concentration (MLVSS), as the dissolved organics were converted into granules [36].

Different OLRs (12.5–55.5 g-COD/l d) were applied at different days of POME fermentation. As the reactor R2–R6 approached its maximum operational limit, the COD removal rate reached a maximum and then decreased, indicating that insufficient microbial biomass aggregation in the reactors. The average COD removal efficiency declined to 55% and the VFAs in the effluent rose to 200 mg/l after 120 days (Fig. 2). The subsequent decrease in the OLR to 35.5–12.5 g-COD/l d resulted in a rapid recovery of the system, revealed by a COD removal efficiency of 93.7% and significant reductions in the effluent VFA (<200 mg/l). The OLR was then kept at 12.5 g-COD/l d to study the UASB performance at higher concentrations of influent COD (days 95–150). It was found that the COD removal efficiency gradually decreased from 91.5% to 72.4% as the concentration of influent COD increased stepwise from 35,000 to 65,000 mg/l. High and consistent concentrations of VFAs in the effluents were observed when the reactor was fed with POME at 45.5 g-COD/l d.

### 3.3. Reactor VSS and MLVSS concentration

Among all the reactors (R1–R6) supplemented with CaO–CKD, R2 provided largest granular size (>0.8 mm) within 30 days of operation, whereas the granular sizes in the reactor without the CaO supplement (R1) became visible after 60 days. Moreover, during this experiment R1, R2–R6 could reach steady state within 60–90 days, respectively. The VSS profile in the effluent indicates the reactor sludge retention of biomass at a given OLR. Fig. 3 shows the VSS profile in the effluent at an OLR of 12.5 g-COD/l d (Fig. 3). Initially because of poor settleability of biomass, more biomass wash out from the reactors R2–R6 was observed. This was further reflected by an increase in effluent VSS concentration at initial stages. After that, the progressive improvement of biomass settleability along with gradual decreased of effluent VSS, indicated a good sign of biomass granulation. Granulation was observed on day 45 for R2–R6, the effluent VSS concentration decreased considerably. A considerable increase in volatile solid concentration and MLVSS in the reactor indicated the development of active biomass from POME (Fig. 3).

The accumulation of biomass for the CaO–CKD dosages on day 150 is shown in Table 3. The MLVSS and SVI concentrations during the accumulation and biomass build-up phase while treating POME are shown in Table 4. During the accumulation phase the SVI

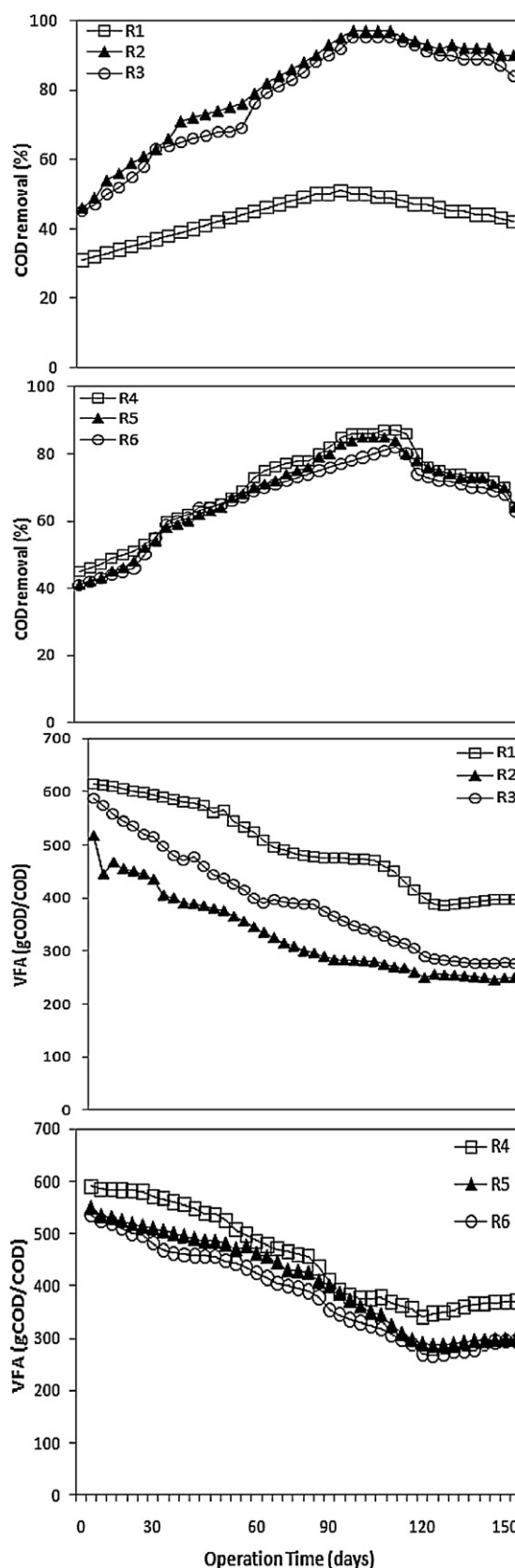


Fig. 2. Operational parameters and performance of the UASB reactors at various doses of CaO: COD removal efficiencies and VFA concentrations of R1–R6.



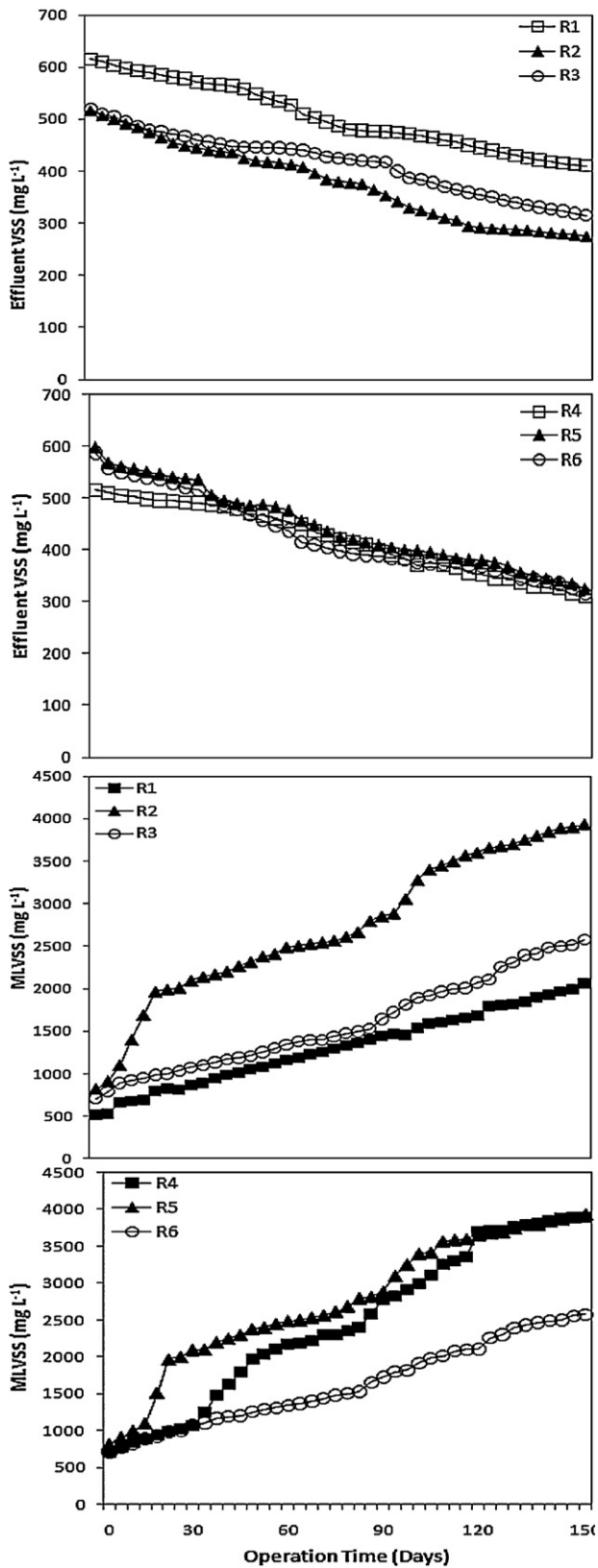


Fig. 3. Operational parameters and performance of the UASB reactors at various doses of CaO: effluent VSS concentrations and MLVSS concentrations of R1–R3 and R4–R6.

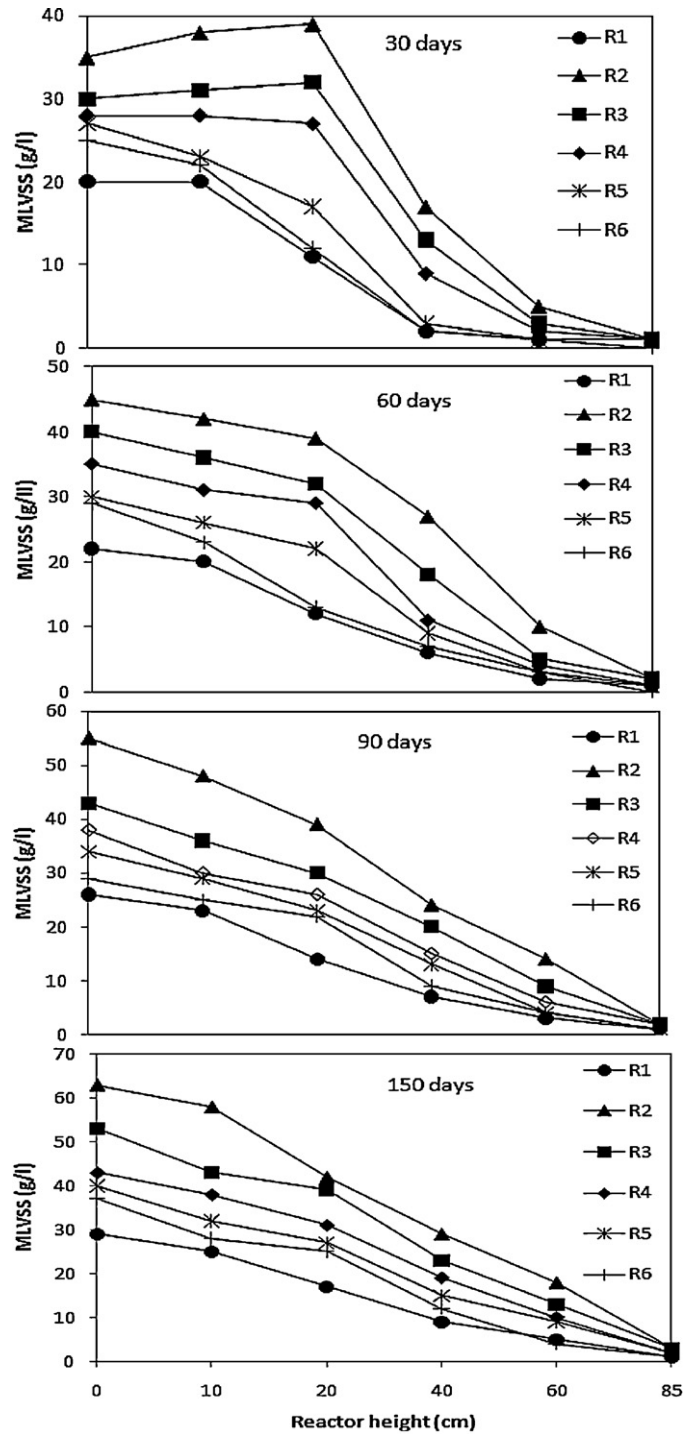


Fig. 4. Biomass development during different days of start-up and UASB reactor height.

and MLVSS steadily rose and reached to 209 mg/g and 40,000 mg/l, respectively. With the development of granulation, the biomass progressively separated with the granules and settled in lower part of the reactor reaction zone. Moreover, the biomass exhibited good settling characteristics, as the MLSS (5500 mg/l) and SVI increased with increasing dosages of CaO–CKD (Table 4). The presence of CaO–CKD in the reactor improved the biomass, while higher dosages of CaO–CKD did not significantly enhance biomass (active biomass–MLVSS) (Fig. 4).

After 45 days of reactor operation, granules with diameters of 0.2–0.6 mm became visible in the lower part of R2. The initial

**Table 5**  
Profile of biomass during various dosage of CaO–CKD in UASB reactors after 150 days.

Ineffluent CaO concentration (g/l)	R1	R2	R3	R4	R5	R6
CaO in granules (mg/l)	950	7050	5920	3739	2780	1350
Dry weight (% of wet sludge)	11.78	32.33	22.50	20.63	19.98	18.50
MLVSS/MLSS	55.3	75.5	72.8	71.2	45.9	29.4
Density of granules (g/l)	1000	1900	1600	1400	1200	1100
Size of the granules (mm)	<0.6	<4.2	<2.6	<2.0	<1.5	<1.5

granules grew rapidly and after 50 days of reactor operation large granules with diameters over 2.0 mm were formed. At day 150, granules larger than 4.0 mm were formed (Table 4). The higher dosages of CaO–CKD and influent OLR (12.5 g-COD/l/d) increased biological growth, granulation, and granule size distribution. Table 5 shows the CaO–CKD concentration in the granules taken from the bottom sampling port of the reactors.

### 3.4. CaO–CKD addition for granulation

Experimental granules with large cavities, likely for escaping biogas, were larger at 90–150 days compared to the control. Calcium carbonate and hydroxide, which precipitated phenolic compounds and long chain fatty acids that were toxic to methanogenic bacteria, improved the total alkalinity and caused beneficial adjustments of pH in POME [14]. Indeed, pH adjustment of fermented POME to 7.5 by CaO–CKD, enhanced the elimination and degradation of the residual complex compounds and volatile fatty acids (Fig. 2). Extensive polymerization of carbohydrates by calcium oxide at pH 7.5 enhances their elimination and completely detoxifies aqueous spruce bark [37].

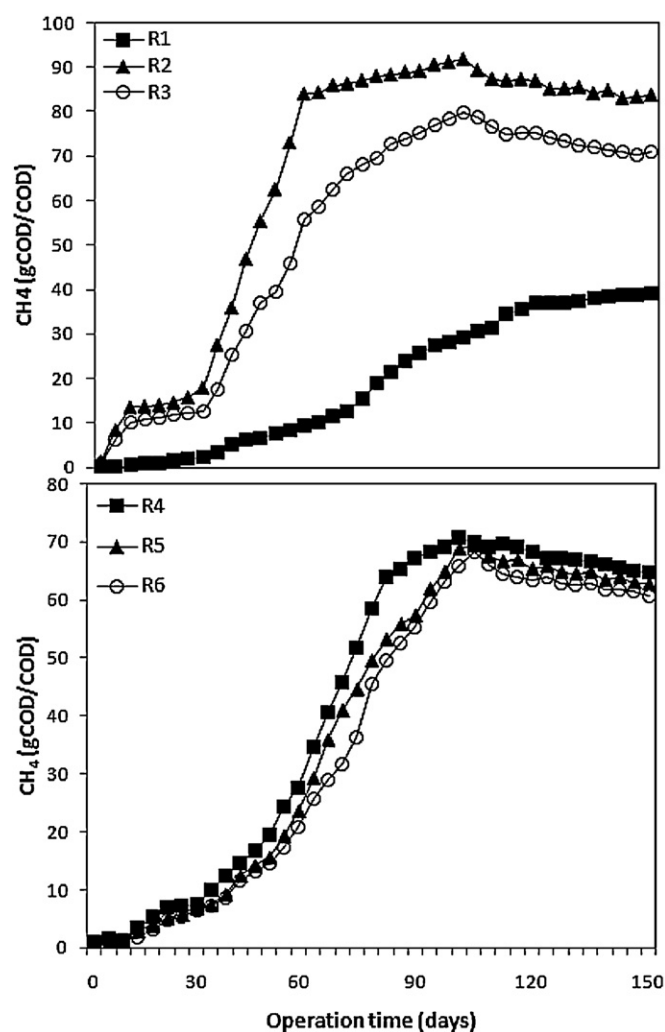
One gram of granular sludge organic matter (dry weight) can catalyze the conversion of 0.5–1 g-COD per day to methane [38]. As shown in Table 5, the granules contained  $32.3 \pm 4.4\%$  dried mass. When the CaO–CKD concentration in the substrate was raised, the water content of the granules decreased and the total dry mass increased. In the dry materials, the proportion of minerals increased significantly while that of organics reduced as indicated by the decrease in MLVSS/MLSS (Table 4). This indicated that the presence of CaO–CKD increased the dry mass of the granules mainly by increasing the concentration of minerals in the granules. The increased mineral content was likely the result of more calcium hydroxide, oxides of carbonates, and aluminum precipitates being trapped in the granules. The density of the granules also increased with increasing CaO–CKD concentration in the feed (Table 5). Fig. 6a illustrates the spatial and porous arrangement of the surface of granules.

Thus, CaO in the process of granulation in UASB reactors could play a significant role at various steps, including (a) during the migration of cells to the surface of other cells, (b) increasing precipitation by physicochemical forces, (c) enhancing adhesion of the cells by microbial cavities or polymers and (d) the multiplication of cells for the development of granules [2,9]. In this study, as shown in Figs. 6 and 7, granular growth and granular cavities developed faster in the CaO–CKD added reactors compared to control reactor (Fig. 7a).

### 3.5. SMA activity and biogas

Table 5 illustrates the SMA activities of the granular sludge, on days 30, 90, 120, and 150, using butyrate as an energy substrate. The methanogenic activity of the granules increased with increasing OLR for all the reactors at the initial stage [39]. At the end of experiment, the SMA of R1 was 1150 mg-CH<sub>4</sub>-COD/g-VSS d which was lower than R4–R6. Reactor R2 was at 1950 mg-CH<sub>4</sub>-COD/g-VSS d throughout the experiment. In addition, it has previously been

reported that the SMA of the sludge decreases and the mass transfer becomes low with severe calcium oxide precipitation in the granules [38,40]. Thus, the CaO–CKD precipitation in the sludge could be due the formation of calcium carbonate or calcium hydroxide. When the OLR was increased up to 12.0–35.0 g-COD/l/d, the SMA in all reactors in the early days increased and then decreased in the final days. When the OLR was further increased to 55.5 g-COD/l/d from day 130 to 150, the performance of the reactor deteriorated considerably, with the sharp drop in COD removal efficiency (66.9%) along with reduction of methane content in the biogas (Fig. 2 and Fig. 5). The granules at high CaO–CKD concentrations had lower mean SMA (metabolic activity). The concentration of CaO–CKD in the granules taken from the bottom of the reactor on day 150 was nearly equal to the substrate. Calcium oxide accumulation within the granules resulted in the interaction of oxide ions with carbonate and exo-polysaccharide polymers.



**Fig. 5.** Operational parameters and performance of the UASB reactors: CH<sub>4</sub> production concentrations of R1–R3 and R4–R6.

The disparities found regarding the effect of CaO–CKD on granulation can be resolved. In this study, we found a beneficial effect of CaO–CKD on granulation and that the granules had large cavities, likely for biogas to escape, as shown in Fig. 7 (a–d). The addition of high dosage of CaO–CKD was found to be detrimental to granulation. Addition of 10 g/l of CaO–CKD showed bigger biogas cavities for more gas to escape after 150 days compared to biogas cavities after 90 days (Fig. 7) less escape than 150 days was showing bigger escape. The present study clearly demonstrates that 10 g/l of CaO–CKD had a positive effect on the sludge granulation process and high calcium concentrations (20 g/l) had a negative effect on granulation. This is in agreement with the results of various researchers (Table 6).

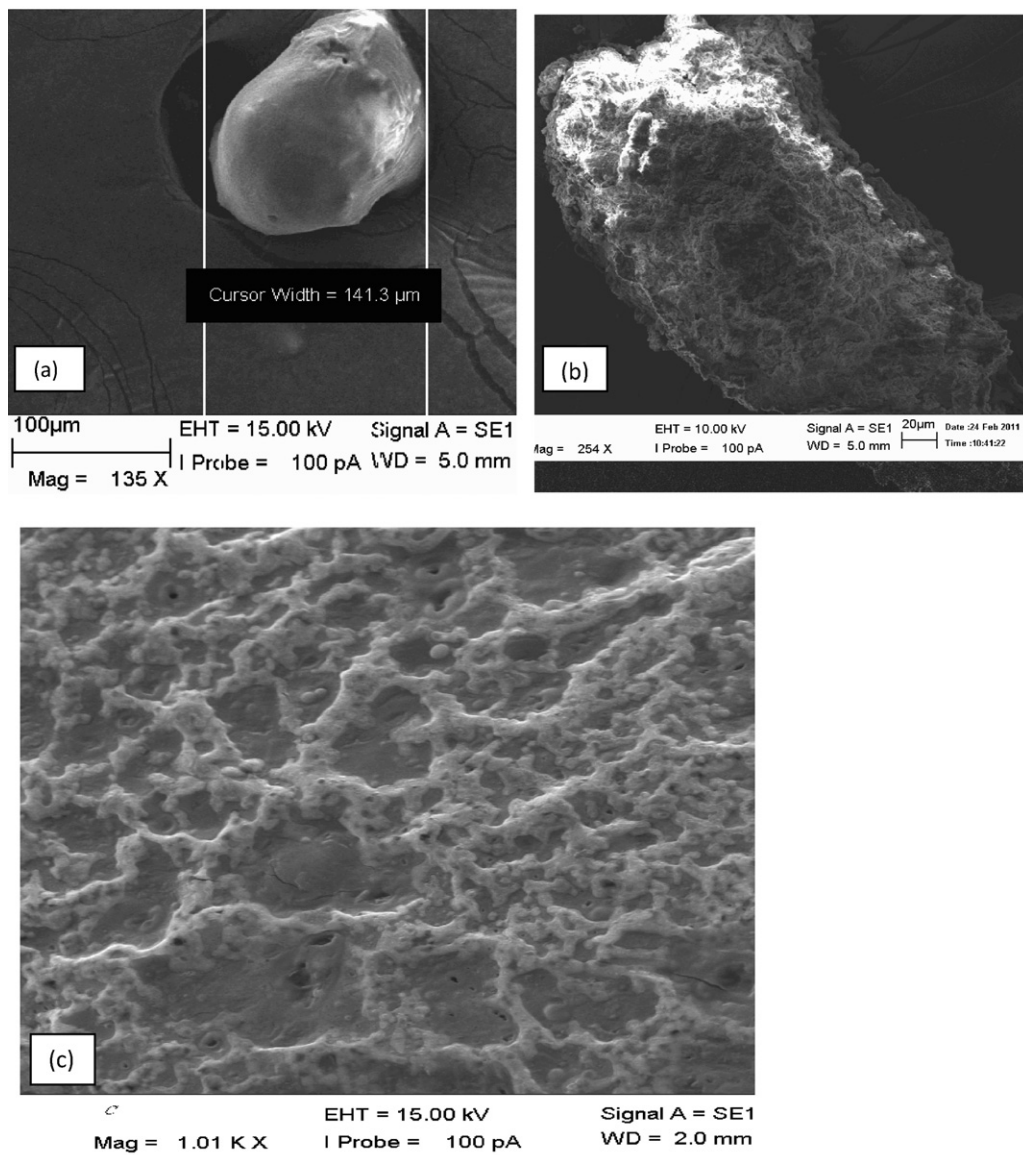
Methane production rate ( $\text{m}^3/\text{m}^3 \text{ d}$ , per  $\text{m}^3$  of the reactor per day) in all the reactors (R1–R6) is shown in Fig. 5. Methane production increased with increasing influent COD concentration. The production in R1 was significantly lower than in R2–R6. Before and after the circulation from day 90 to day 96 (under the same COD concentration of 12.5 g/l), the methane production in R2 increased significantly from  $1.19 \text{ m}^3/\text{m}^3 \text{ d}$  to  $1.51 \text{ m}^3/\text{m}^3 \text{ d}$ ,

**Table 6**

Effect of (10 g/l CaO) on SMA ( $\text{mg-CH}_4\text{-COD}/\text{mg-VSS d}$ ) using butyrate as substrate for energy sources for the growth of biomass in the UASB reactor.

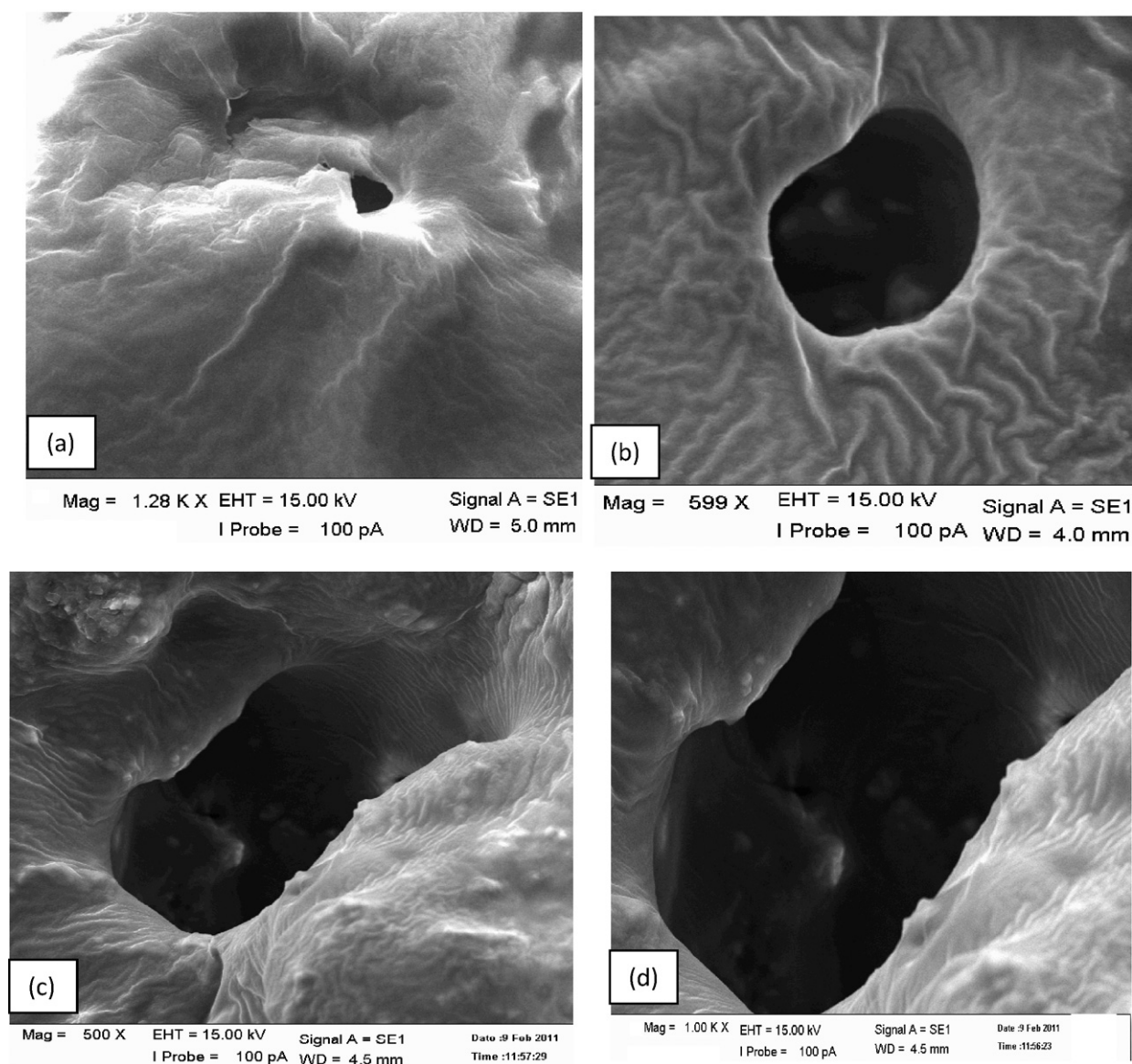
Reactor	Fermentation time (d)					
	0	30	60	90	120	150
R1	360	800	990	1050	1100	1150
R2	380	1050	1290	1480	1789	1950
R3	370	950	1070	1080	1160	1350
R4	370	920	1170	1290	980	1180
R5	360	950	1080	1050	1130	1170
R6	350	900	990	1040	1120	1160

compared to  $0.99 \text{ m}^3/\text{m}^3 \text{ d}$  to  $1.07 \text{ m}^3/\text{m}^3 \text{ d}$  in R1. This indicates that circulation could effectively enhance methanogenic activity in R2 and was consistent as shown in Fig. 5. The content of  $\text{CH}_4$  in the R2 biogas increased from 40.2% to 48.2% when circulation was applied. The methane production per gram of COD removed amounted to 320 ml, which was close to the theoretical  $\text{CH}_4$  production from 1 g COD, i.e., 350 ml  $\text{CH}_4/\text{g-COD}$  removed [41].



**Fig. 6.** (a) SEM micrograph of granules; (b) outer surface of the granule; (c) scanning electron micrographs of the granule: Archaea (*Methanosarcina* sp.) showing the arrangement of bacterial cells in granule surrounded by extracellular polymeric substances (EPS), the seed sludge and granules sampled on day 150.





**Fig. 7.** SEM micrographs are showing smooth surface of granule with a large opening cavities likely for biogas escape (a) control WD 4.0 mm, scale 200  $\mu\text{m}$ ; (b) WD 4.0 mm, scale 200  $\mu\text{m}$  at 60 days; (c) (WD 4.5 mm, scale 200  $\mu\text{m}$  at 90 days); and (d) (WD 4.5 mm, scale 200  $\mu\text{m}$  at 150 days).

### 3.6. Scanning electron microscope observations

The reactor stability can be observed by monitoring the key parameters of reactor i.e. stable pH, effluent VFA, and MLVSS development. The performance of the reactors, in terms of COD removal efficiency, effluent VSS concentration, granulation and methane production, continuously improved with the operation time. Scanning electron microscope observations revealed that the sludge from R2 to R6 had similar composition and morphology. The granules from the six reactors were predominantly composed of *Methanosarcina*-like species that appeared packed in the core of developed granules (Fig. 6c) [42]. This implies that the addition of CaO-CKD at 10 g/l did not result any difference in the predominant microorganisms, despite the observation that it accelerated the granulation process and led to the formation of larger sized granules. The texture (surface topography) of the granules observed from the methanogenic dominant zone (bottom zone) was more regular, densely packed, with an outer, rigid, and stable structure and smooth parts with a rough exterior surface (Fig. 6 a–c). It was previously reported that anaerobic granules in methanogenic

dominant zone exhibited fissures, a less stable structure and broken parts with a rough exterior surface [37]. According to the description of granular structure [32], acidogenic organisms were predominant in the outer layer of the anaerobic granule, which protected the methanogens in the core layer from exposure to oxygen and low pH. Scanning electron microscope observation revealed that the sludge from R1 had a very similar composition to that from R2, but the morphology was different. The granules from both reactors were predominantly composed of *Methanosarcina*-like species. A network of *Methanosarcina*-like species was observed in the core zone (Fig. 7b–d) compared to the control along with large number of cavities (Fig. 6c). Extracellular polymeric substances (EPS) were observed within the entire granular structure that made an interlinked network and a large number of bacterial cells were surrounded by EPS (Fig. 6c).

The comparison of present study to the previous studies on the effect of CaO-CKD on sludge granulation shows that CaO-CKD has a positive effect very similar to  $\text{Ca}^{2+}$  and  $\text{Fe}^{2+}$  [5,9,10]. These ions caused granules to form earlier, grow larger and resulted in a faster granulation process with a short start-up period of UASB



reactors. Also, the addition of these ions did not lead to a difference in the predominant microorganisms. Therefore, it seems that CaO–CKD enhances the sludge granulation process through the same physicochemical function. However, when CaO is added, mineral precipitates such as  $\text{CaCO}_3$  and  $\text{Ca}_5\text{OH}(\text{PO}_4)_3$  are formed and there is a risk of cementation of the UASB reactors [41]. The same is true for the formation of FeS when  $\text{Fe}^{2+}$  is added to UASB reactors. On the other hand, there is no such a risk when CaO–CKD is used. Therefore, CaO–CKD might be a better inexpensive option than  $\text{Ca}^{2+}$ ,  $\text{Al}^{3+}$ , and  $\text{Fe}^{2+}$  for enhancing sludge granulation in UASB reactors.

#### 4. Conclusions

The results and discussion of this work indicate that calcium oxide is feasible for POME treatment. An average COD removal efficiency of 82.4% was achieved when a reactor was fed with 10 g/l CaO (COD as high as 70,390–75,480 mg/l) at an OLR of 12.5 kg-COD/m<sup>3</sup> d under mesophilic condition. Due to the high CaO concentration, the VS/MLVSS ratio of the sludge decreased significantly after long-term operation with the precipitation of calcium carbonate. The average methane yield was 0.91 l  $\text{CH}_4$ /g-COD removed and 88.6% of the removed COD was converted to methane with an OLR of 12.5 kg-COD/m<sup>3</sup> d. The specific activity of the granules decreased with an increasing calcium oxide concentration in the feed. Thus, along with the significant decrease of water content in granules and the toxicity of high-concentration calcium oxide that accumulated inside the granules caused a lower bacterial specific activity.

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