*Desalination and Water Treatment*
www.deswater.com

doi: 10.5004/dwt.2022.28268

252 (2022) 89–97
March

# Natural gum based polymers as additives in enhancing the startup of UASB reactor treating municipal sewage — comparative study

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Received 25 October 2021; Accepted 3 February 2022

**ABSTRACT**

This work has focused on the potential of natural gum based polymers as additives, to enhance the startup of upflow anaerobic sludge blanket (UASB) reactors treating municipal sewage. A laboratory study was taken up to substantiate this novel idea. Under identical conditions 4 similar UASB reactors were operated in parallel. Reactors locust bean gum (LBG) and guar gum (GG) were injected with the novel bio-flocculants LBG and guar gum. For better insights their performance was compared with the well proven cationic polymer chitosan, in reactor (CH). Reactor (CT) without any additive served as control. Reactor with LBG as additive outclassed others with an accelerated startup in 40 d reaching a maximum chemical oxygen demand removal of 92.5%, followed by GG, CH and CT with 88%, 80% and 60% removal respectively at the end of study period. Both LBG and GG also succeeded with a dense sludge bed anchoring most of the anaerobes denoted by a least sludge volume index of 26.2 and 29 mL/g, followed by high volatile suspended solids/total suspended solids ratio of 0.75 and 0.72, respectively. Specific methanogenic activity and ECP secretions of all 4 reactors were almost similar, indicating that early granulation was primarily induced by the polymers. Hence in a nut shell, polymer enhanced reactors visualized an accelerated startup along with better biomass retention than the control.

*Keywords:* Anaerobic granulation; Guar gum; Locust bean gum; Municipal sewage; Startup-time; Upflow anaerobic sludge blanket reactor

## 1. Introduction

With the growing population one common issue faced by almost all developing countries is the proper treatment and discharge of municipal wastewaters. Municipal sewage with chemical oxygen demand (COD) less than 1,500–2,000 mg/L are seldom given complete treatment before being discharged, thus polluting the water bodies. This can be attributed to the high cost and maintenance of treatment systems and also to the availability of land in urban areas. As the conservation of water bodies and aquatic ecosystem has risen as the need of the hour, there comes the dire need for a treatment scheme with the following features: simpler design, ease to construction and maintenance, reduced carbon footprints and high treatment efficiency [1]. The anaerobic technology offer great potential in this regard, as they have transformed the treatment plants from energy consuming to energy producing systems [2].

Of all the anaerobic systems, granule based upflow anaerobic sludge blanket (UASB) reactors are preferred for their high treatment potential. A granule, is a typical aggregation of biomass which forms the soul of this treatment process. These granules have enhanced settleability, which prevent the washout of biomass and are also capable of providing intimate contact between the microbes and organic matter, which further enhances the treatment [3,4]. However the major shortfall of UASB reactor is its extremely long startup phase, that is, it takes nearly 3–8 months for the

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slow growing anaerobes to granulate [5]. To overcome this limitation and to extend the application of UASB reactors, a wide number of studies have been conducted.

Usage of polymers to enhance the startup, by immobilizing the biomass and reinforcing the strength of mature granules is one method which has been practiced for years [6]. Natural polymers such as chitosan, Reetha extract [7], water extract of *Moringa oleifera* seeds (WEMOS) [8], powdered bamboo charcoal [9] and synthetic polymers like AA184H [10] and Percol 763 [11] have shown promising results in enhancing the startup of UASB reactors. These polymers because of their polysaccharidic structure much similar to extracellular polymeric substances (EPS), accelerate granulation in the initial stages through the adhesion of dispersed microbes [12,13]. Similarly ECP secreted by the anaerobic microbes, are good at bridging the bacterial cells physically and binding them to inert substances [14]. Yet ECP secretion is much more influenced by the type and composition of wastewater and also by the operational conditions. Studies suggest that the addition of multivalent cations such as $Al^{3+}$, $Fe^{2+}$ and $Ca^{2+}$ at optimum concentrations have enhanced granulation [15–17]. Support materials like powdered activated carbon, diatomite, maifanite and turf soil have also improved granulation, by their large surface area available for microbial attachment and aggregation [18,19]. However most of these findings are limited to high strength wastewaters.

When it comes to low strength wastewaters, especially municipal sewage UASB reactors lack with high treatment efficiency. Municipal sewage is often characterized with a high suspended solids content, which lengthens the hydrolysis phase. Also with a low organic content the biogas production is also considerably reduced, thereby leading to poor mixing and formation of localized channels in the sludge bed [20]. Hence this study is mainly aimed to reduce both the shortfalls of UASB systems, that is, enhancing the startup of UASB reactors treating low strength municipal sewage by the addition of a novel bio-flocculent.

Guar gum (GG) and locust bean gum (LBG) are the two novel natural non-ionic polymers introduced in this study, to enhance the startup. Both are water-based viscosifiers with exotic self-gelling property and coagulating potential, which has been the reason behind selection of these 2 polymers. LBG is a creamy white powder obtained by grinding the seed endosperm of carob tree (*Ceratonia siliqua)* and GG is got from the endosperm of guar plant (*Cyamopsis tetragonolobus).* GG and LBG are composed mainly of the complex carbohydrate polymer of galactose and mannose in different proportions [21,22]. Their polysaccharidic structure facilitates the aggregation of anaerobic sludge through hydrogen bonding and the viscosity created by these polymers comes as an added benefit in enhancing microbial contact and attachment. Being non-ionic their solutions are also not influenced by pH, salts and heat treatment. As the additives chosen for this study are bio-polymers they possess the ease to degradation and cause no harm to the environment [23]. For better validation their ability is to be compared with the cationic polymer chitosan – which has been used for decades to accelerate granulation.

Hence a comparative study on enhancement of startup phase using GG, LBG and chitosan was carried out during the treatment of municipal sewage. Effluent and sludge characteristics where analyzed in detail, to pin down the best suitable polymer for shortening the startup phase on treatment of low strength wastewater. Since this study is carried out with actual wastewater rather than simulated wastewater, it would be easier to scale up for field applications.

## 2. Materials and methods

### 2.1. Reactor configuration

Experiments were done in parallel in 4 identical reactors each of (2 L) capacity, for 60 d at room temperature. Sludge samples for analysis were drawn from the port at the bottom of the reactor. The wastewater for the study was collected from the sewage treatment plant (STP) at Anna University, Chennai – India. The raw sewage collected after screening, was characterized for the qualitative and quantitative parameters following (APHA 2005 standards) and presented in Table 1 [24].

### 2.2. Seed sludge

The seed sludge was obtained from the anaerobic digester of an STP at Perungudi – Chennai – India. Prior to seeding the sludge was screened through a 0.15 mm sieve to remove the floating debris. Detailed characteristics of the seed sludge are as presented in Table 2. Each reactor was fed with the seed sludge to one third of its capacity.

### 2.3. Dose of additives

For the ease of description, three reactors started with the additives locust bean gum, guar gum and Chitosan

Table 1
Characteristics of raw sewage fed to the reactors

| Parameters | Value |
|---|---|
| pH | 6.8–7.2 |
| Biochemical oxygen demand | 330–375 (mg/L) |
| Chemical oxygen demand | 750–880 (mg/L) |
| Total suspended solids | 370–392 (mg/L) |
| Volatile suspended solids | 330–354 (mg/L) |
| Alkalinity | 300–330 (mg/L as $CaCO_3$) |
| Volatile fatty acids | 12–18 (mg/L) |
| Total Kjeldahl nitrogen | 34–45 (mg/L) |
| Total phosphate as P | 20–30 (mg/L) |
| Sulphate as $SO_4$ | 55–68 (mg/L) |

Table 2
Characteristics of seed sludge

| Parameters | Value |
|---|---|
| MLSS | 12,070–12,125 (mg/L) |
| MLVSS | 6,182–6,230 (mg/L) |
| SVI | 51–53 (mL/g) |
| VSS/TSS | 0.50–0.51 |
| SMA | 0.155–0.168 (g $CH_4$-COD/g VSS/d) |

were named as LBG, GG and CH respectively. Whereas the control reactor without any additive was named as CT. LBG with an average molecular weight of $1.5 \times 10^6$ Da was supplied by courtesy of IAMPURE Ingredients, India and GG of molecular weight $2.2 \times 10^6$ Da was procured from Istore Direct Trading Pvt. Ltd. India. Chitosan obtained by (≈90% deacetylation) of chitin, with an average molecular weight of $1.8 \times 10^5$ Da was kindly provided by Mahtani Chitosan Pvt. Ltd., Veraval, India.

Dosage of additives at the startup was assessed by measuring the maximum turbidity removal efficiency and the minimum sludge volume index (SVI) of the flocculated biomass using Jar test method [25]. Chitosan stock solution was prepared by mixing 1 g of chitosan in 100 ml of 1% acetic acid at 200 rpm for 24 h. Fresh stock solutions of both the biopolymers was prepared to a concentration of 1 g/L at the start of each test, to avoid the growth of molds. Sludge samples with additives of varying concentrations were mixed by an (Eie-109 Digital Jar test apparatus) in 2 stages: flash mixing at 150 rpm for 5 min, followed by slow mixing at 50 rpm for 15 min. After mixing they were left undisturbed for 30 min and subsequently turbidity of the supernatant liquid was assessed by (METZ 501M digital turbidity meter). The concentration which yielded maximum turbidity removal was chosen as optimum dose of that polymer. Fig. 1 illustrates the outcomes of Jar test – turbidity removal efficiencies of the chosen additives at varying concentrations.

The concentrations so found, in (mg of the additive/g of total suspended solids of the sludge) added to the respective reactors were, locust bean gum 20 mg, guar gum 18.5 mg and chitosan 25 mg respectively. At the startup the additives were initially dissolved in 100 mL of distilled water and then mixed with the seed sludge. The seed sludge with the added additives was stirred at 200 rpm for 5 min, followed by a constant stirring at 100 rpm for 12 h. This aided in complete mixing of polymers with the seed sludge, followed by gradual aggregation of the biomass and thereby paving the way for granulation. Interim dose of polymers was diluted with 100 mL of distilled water and fed to the reactors, followed by a standstill period of 3 h to ensure good sorption of the additives to the sludge bed. A total of 5 such dosages was given at an interval of 10 d, for the entire study period.

### *2.4. Analytical method*

Influent and the effluent samples from each of the reactors was analyzed for pH, alkalinity, COD, volatile suspended solids (VSS) (APHA Standards 2005) and volatile fatty acid (VFA) on every alternate day [24,26]. The pH of influent and effluent samples was determined using a digital pH meter (manufactured by Vani International, India). Closed reflux colorimetric method was adopted for estimation of COD. VFA concentration was assessed by titration method following (DiLallo and Albertson1961) – first the samples were titrated as a part of alkalinity method, boiled to remove excess $CO_2$ and then back titrated to a pH of 7 [26]. The sludge samples taken from the bottom of each reactor, was analyzed for mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS) and SVI as per (APHA Standards 2005) on every alternate day [24].

Visual examination of the granules was done periodically followed by scanning electron microscopy (SEM) to study their surface morphology. The sludge samples were prepared for analysis by fixing it in a 2.5% glutaraldehyde solution, dehydrating in water – ethanol solutions, followed by drying to the critical point and finally sputter coating it with gold. SEM images were then taken with a JEOL JSM-500LV microscope.

ECP content comprising of (polysaccharides and protein) plays an integral part in the granulation process, hence it was also quantified. The cooling extraction method as described below was adopted for the extraction of ECP

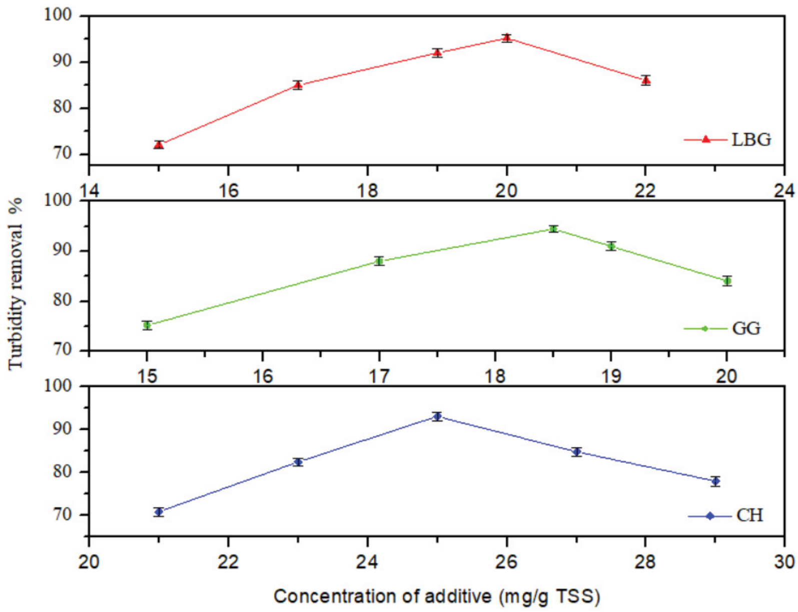


Fig. 1. Turbidity removal efficiencies of the chosen additives at varied concentrations.

from the sludge [27]. 2 mL of sludge sample was taken for analysis and centrifuged to remove the supernatant. This was followed by the addition of 10 mL of 0.85% NaCl and 60 mL of formalin. This mixed liquor was placed in an ultrasonicator for 300 s while being cooled in ice water for the extraction of ECP content. The mixture was then centrifuged at 12,000 rpm for 30 min and the supernatant was separated and analyzed for polysaccharide and protein. Sulfuric acid-anthrone method was followed for polysaccharide analysis and Lowry Folin method helped with protein quantification [28].

The maximum specific methanogenic activity (SMA) was determined by serum bottle technique, using sodium acetate as the sole substrate at 35°C + 1°C under anaerobic conditions [29]. As time elapsed, concentration of substrate from the serum bottle was assessed. Maximum slope of the concentration curve plotted, indicated the activity of methanogenic bacteria.

## 3. Results and discussion

### 3.1. Reactor performance start-up phase

All four reactors were started with an organic loading rate (OLR) of 1 kg COD/m$^3$/d and hydraulic retention time of (HRT) of 24 h, which was maintained for the entire study period. The reactor's performance in terms of COD removal efficiency is illustrated as in Fig. 2. For the first 15 d COD removal efficiencies of all four reactors where mostly similar. This can be considered as the acclimatization period for the microbes. On further precedence, with the active growth of microbes the removal efficiency of polymer enhanced reactors increased by folds higher than the control. As the polymers promoted bacterial agglomeration more of biomass was retained in the system, which aided in active degradation of organic matter. LBG with a highest removal of 92.5% on the 40th day marked a successful completion of startup phase. Fig. 9a portrays the tiny granules that have emerged at the end of 40th day in LBG. The reason for enhanced startup can be related to the long chain length of LBG polymer, which supported microbial bridging and its viscous nature promoted the contact between microbes [21]. Due to proximity the formation of granules also enhanced mass transfer between syntrophic groups, resulting in improved removal of organic matter [23]. Removal efficiencies of GG (88%) and CH (78%) where comparatively lesser than LBG (92.5%), yet where higher than CT (60%). These findings can be correlated to biomass content of the systems.

Fig. 2 also interprets the variation of acclimatization, growth and steady phases of all 4 reactors during the startup. This can help us with interlinking the influence of polymers (on growth and retention of biomass), to the enhancement of startup phase. Acclimatization period was similar for all 4 reactors. Growth phase of LBG and GG was steeper with a maximum COD removal of nearly 90%, which was steadily maintained till the end of study period. This can be attributed to the surface properties of the additives LBG and GG – characterized with a rough and porous surface, they were bound to have a high surface area which served as adsorption site for the microbes [30]. Thereby anchoring anaerobes within the system for a longer time has reflected on improved removal efficiency within a shorter span [7]. CH had a gradual rise in growth phase, indicating a longer startup time (52 d) than LBG and GG. The flatter growth phase of CT, implied that the system is in need of more time to achieve the startup.

Fig. 3 illustrates a comparison of VSS concentration in the effluent of all four reactors. This parameter can give us an indication of sludge settleability and the formation of a dense sludge bed [17]. Initially a higher VSS concentration of 180–190 mg/L was observed in the effluent of all 4 reactors, owing to poor settleability of the sludge. With the course of time the effluent VSS concentration in LBG, GG and CH decreased at a faster rate reaching 55, 60 and 72 mg/L at the end of startup phase. This proves the efficacy of polymers in improving the settleability of biomass. While CT with 132 mg/L of VSS on the 40th day, implied that the system is yet to develop a dense sludge bed.

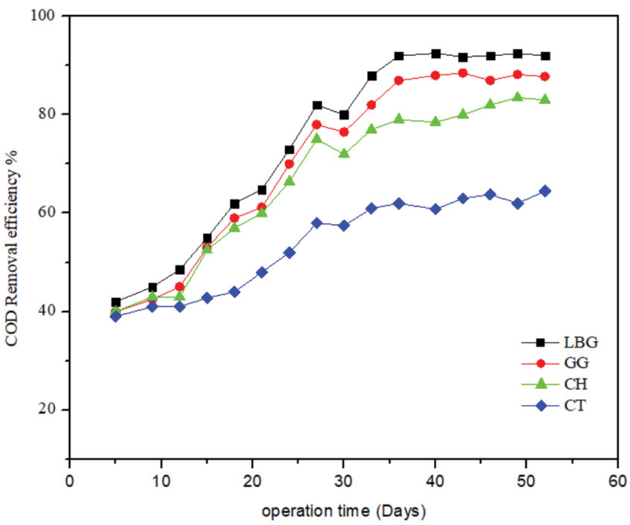


Fig. 2. COD removal efficiency for the startup phase.

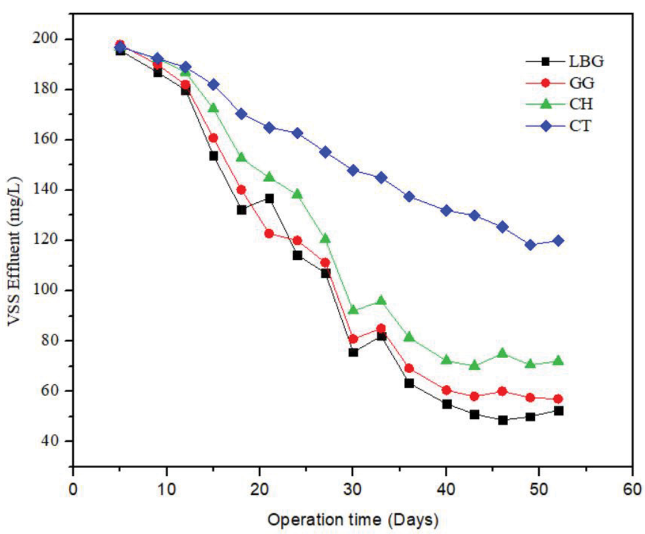


Fig. 3. Comparison of VSS concentration in the effluent.

The pH of the feed and effluents were regularly monitored for the entire study period as depicted in Fig. 4. It has been observed that, the pH in all three reactors namely LBG, GG and CH was found to be slightly acidic (6.6–6.8) for the first 15 d indicating the prevalence of hydrolysis over methanogenesis. The COD removal efficiency during this period was also found to be low. After the 15th day a marginal rise in pH trend (6.9–7.3) was observed in LBG, GG and CH due to the stabilization of excess VFA by microorganisms – followed by an increased removal efficiency. This can be taken as an indication of successful startup in terms of pH. In the case of CT there was a slight decline in pH during the initial stages which then started to rise at a slower rate than the polymer enhanced systems. Alkalinity changes were also similar to that of pH.

As the fluctuations in VFA concentration manifests the activity of methanogens and treatment potential of a system, it was also quantified for better validation of the process. Fig. 5 portrays a comparison of alkalinity and VFA in the effluent of all 4 reactors during the startup phase. Initially an increase in VFA concentration (20–39 mg/L)

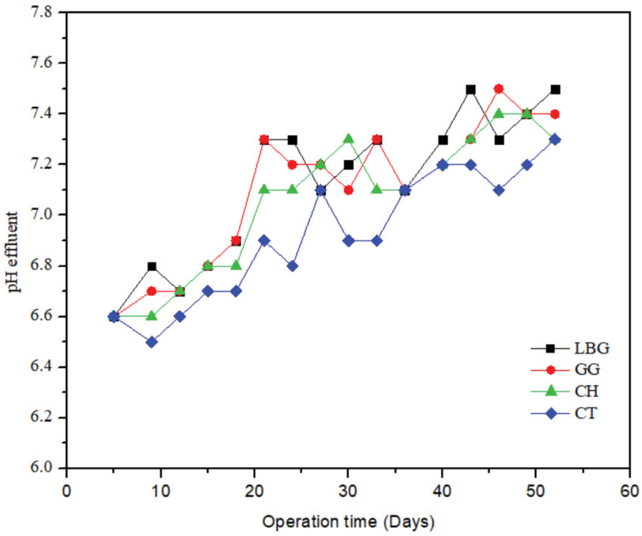


Fig. 4. Variation of effluent pH for various reactors.

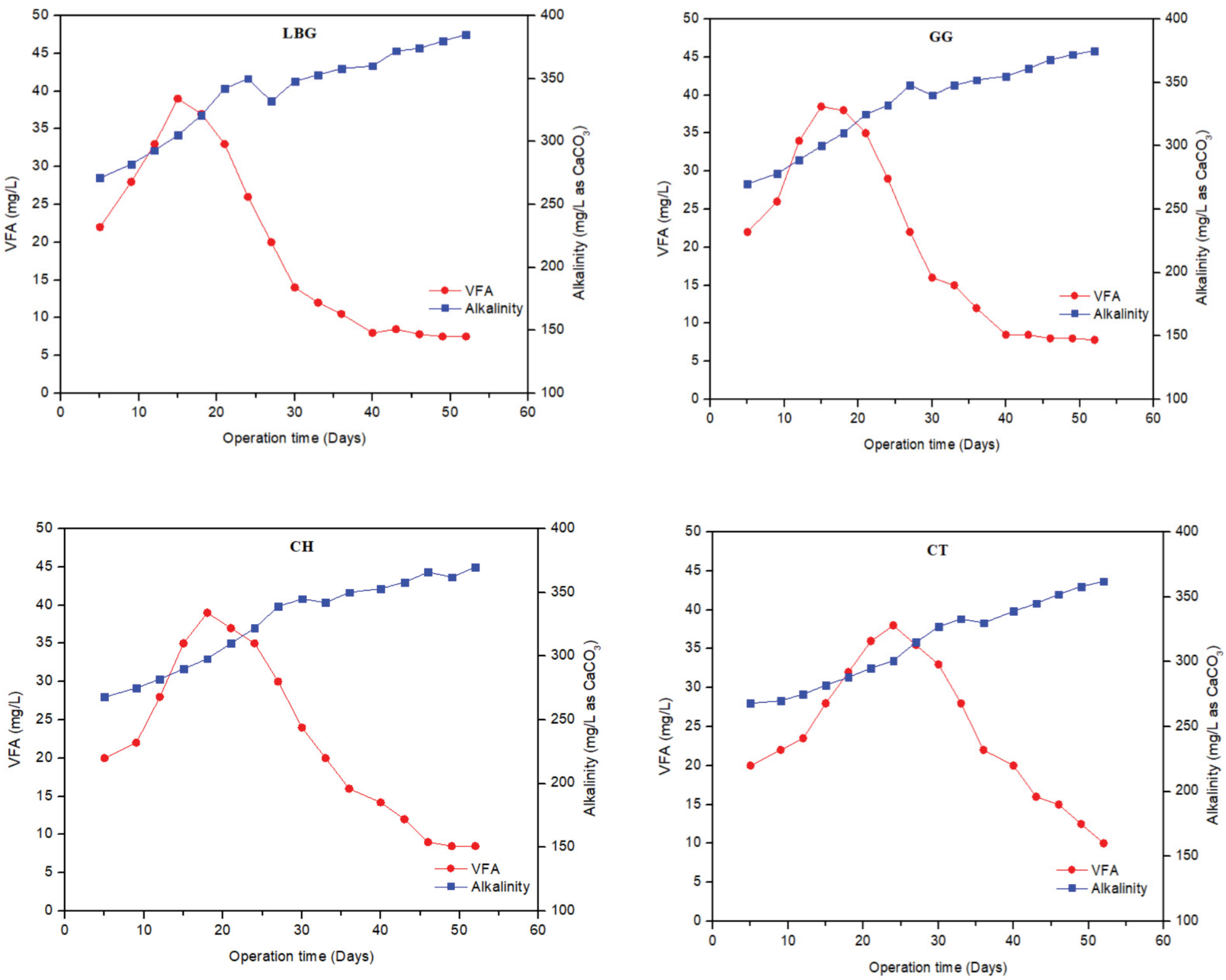


Fig. 5. Interplay of alkalinity and VFA for LBG, GG, CH and CT reactors – startup phase.

was observed in all systems as the rate of consumption of volatile fatty acids by microbes was less and moreover due to the lack of equilibrium between acidogens and methanogens. This was also in line with reduced alkalinity (270–300 mg/L) and pH (6.6–6.8) during this phase. As the time progressed VFA concentration in LBG, GG and CH dropped to 8, 8.5 and 14 mg/L indicating the onset of methanogenesis. This period was also observed with an increased alkalinity (350–360 mg/L as $CaCO_3$) and pH (6.9–7.3), which confirmed the success of startup. VFA concentration in CT dropped at a slower rate – which implied a delayed startup of reactor. VFA/alkalinity ratio can be taken as a measure of stability of an anaerobic treatment process, as it indicates the balance between acidogenesis and methanogenesis. All 4 reactors where observed with a ratio in the range of 0.02–0.06 (<0.5) – indication of proper anaerobic functioning [31].

*3.2. Sludge characteristics*

The role of polymer additives in enhancing granulation and thereby shortening the startup phase was validated in terms of sludge settleability, total biomass content and their metabolic activities. These parameters are commonly expressed by SVI, VSS/total suspended solids (TSS) and SMA.

Microbes anchored to the sludge bed are the armed forces of an UASB reactor, taking part in the treatment process. Hence it is mandatory to keep an account of this microbial population. Pictorial representation of the variation of biomass content of all 4 reactors is, as in Fig. 6. A steady increase in VSS/TSS ratio was observed in all reactors with the passage of time, indicating the growth of anaerobic microbes. All 3 reactors with polymer additives had a higher biomass content than the control reactor, for the entire study period. Reactor with LBG as additive visualized a highest ratio of 0.75 at the end of startup phase. While the control reactor (CT) recorded the least biomass content with the ratio of 0.59. This goes by the hypothesis that polymers are effective in retaining the biomass for a longer time within the system [11]. Reactors LBG and GG with the water based viscosifiers newly introduced in this study, accelerated microbial adhesion through hydrogen bonding and controlled sludge washout to a better extent than the natural polymer chitosan (in CH) which has been used for years [32]. Fostering microflora within the sludge bed for a longer time will eventually result in a better removal of organic matter and thereby reducing the startup time. Since the control reactor (CT) couldn't help with the aggregation of biomass, most of the dispersed microbes were washed out of the system. This eventually resulted in a reduced removal of organic matter and a longer time for the system to achieve its startup.

Graphical representation of sludge settleability in terms of SVI for all 4 reactors, is as shown in Fig. 7. The SVI of sludge in all reactors, showed a decreasing trend indicating microbial adhesion and coagulation. Reactors with polymer additives promoted faster aggregation of anaerobic cells to form granules (in line with the findings of EL-Mamouni et al. [11]) and was spotted with a steep decrease in SVI values than reactor CT. The additives used had a better control on microbial washout-indicated by settleability of sludge from the system. A dense sludge bed with good settleability, is the prime seat for the successful operation of an UASB reactor [33]. In case of the control reactor (CT), loose biomass with poor settleability was observed. LBG recorded the least SVI of 26 mL/g at the end of startup phase whereas the control reactor CT was noted with a higher SVI of 43 mL/g. Lower the SVI, higher is the settleability of sludge. The highest sludge settleability in LBG reactor was by the viscosity and gelling nature of the polymer, which aided in the microbial attachment and subsequent flocculation. It was also quite efficient in maintaining its viscous nature over a wide pH range of 4–9 [21]. GG was also noted with a similar trend as LBG with an SVI of 29 mL/g. Though both were water based visocifiers, the viscosity of guar gum was known to decrease with increase in temperature which might have been the reason for difference in performance [22]. Reactor (CH) with the cationic polymer chitosan,

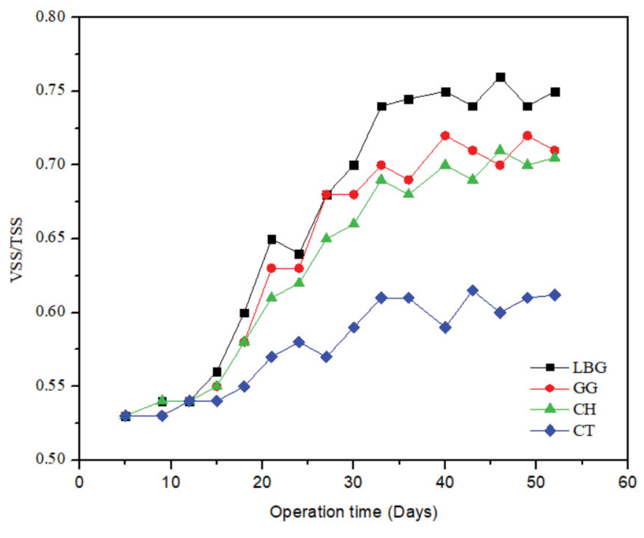


Fig. 6. Comparative study of VSS/TSS ratio of granules from different reactors.

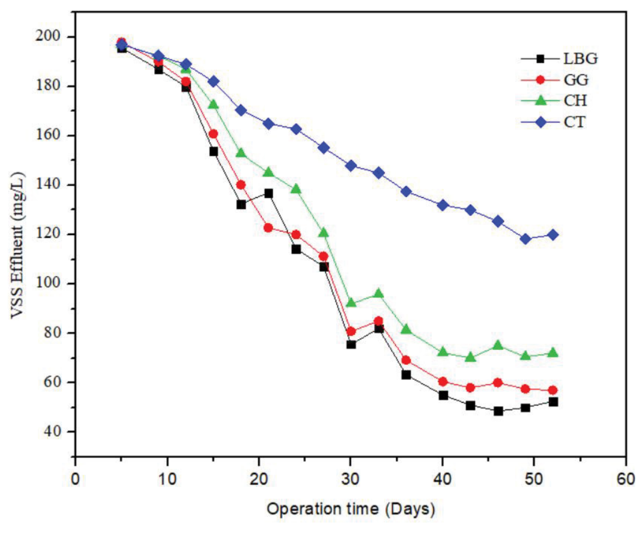


Fig. 7. SVI of sludge from different reactors – startup phase.

recorded an SVI of 33 mL/g which was comparatively better than CT, yet was lower than the performance of novel bio-flocculants used in this study. This can be related to the sensitivity of chitosan to alkaline pH conditions. Where chitosan gets progressively neutralized and becomes less efficient in the flocculation of dispersed sludge particles [11].

Active methanogens in an anaerobic reactor, stand as the critical factor in achieving effective wastewater treatment. Thus quantification of specific methanogenic activity delineates the capability of the seed sludge in producing methane for a specific substrate and stability of the system [34]. The SMA of seed sludge was studied using sodium acetate as the sole substrate, at the beginning and end of startup phase, for all 4 reactors. The methanogenic activity of sludge in the reactors LBG, GG, CH and CT at the end of startup phase was 0.65 ± 0.05, 0.61 ± 0.08, 0.55 ± 0.12 and 0.50 ± 0.05 g $CH_4$-COD/g VSS/d respectively. Only subtle variations were observed in the methanogenic activity of sludge particles, which implied that accelerated startup has resulted only from aggregation and retention of biomass. This further proved that polymers besides enhancing microbial adhesion, had no significant negative effect on the anaerobic microbes. The polymeric layer developed around the cells by the additives, did not inhibit substrate diffusion into and out of the biomass, which was indicated by the SMA results.

ECP (polysaccharide and protein) content was quantified for the sludge samples at the beginning and end of study period as in Fig. 8. All 4 systems had a similar ECP concentration in the range of 1.30–1.40 g/g of VSS at the end of startup phase. Since ECP content is much more related to the type and the quantity of substrate that is supplied, no significant difference was observed in this study. Hence the enhanced startup reported here was primarily influenced by the polymers. Further this confirmed that polymers were effective in enhancing granulation during the early stages which was later supported by ECP secretions [7].

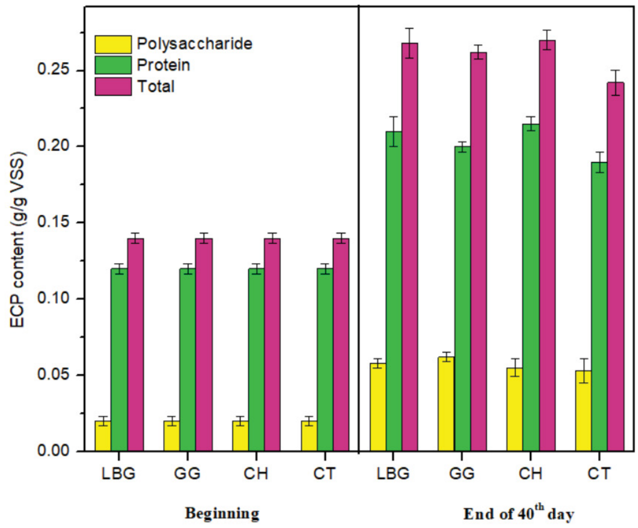


Fig. 8. Variation of ECP content in the sludge at the beginning and end of startup phase.

### 3.3. Morphological examination

Visual examination of granules from reactors LBG, GG and CH (Fig. 9b) revealed a black color of nearly spherical shape with irregular projections on the surface. Morphology of granules was studied by SEM observations for the best performing reactor LBG as in Figs. 9c and d. Outer surface of the granules (Fig. 9c) reveled a rough and uneven texture with tiny sludge particles, which confirmed that granulation has resulted from adhesion of biomass. Similar observations from earlier studies pointed that agglomeration might be a key for better insights to the granulation process [35]. Also randomly distributed cavities on the surface gave us an indication of vigorous biogas production [36]. Fig. 9d portrays micro colonies of rod (*Methanothrix)* and coccus (*Methanosarcina)* type methanogens along with scanty filamentous microbes. Though filamentous bacteria were considered as precursors in granule formation as per the nuclei and multi-layer theory – which stated that first filamentous microbes form a network, to which the cocci and other bacteria gets trapped forming a granule [37,38]. In this study filamentous bacteria has not lead to granulation similar to the findings of Zhou et al. [35]. In spite of a shorter granulation period no significant difference was observed in the microbial phase of granules. This further strengthened the idea of using natural gum based polymers – LBG, in the enhancement of granulation.

## 4. Conclusion

This work explored a new method of using natural gum based polymers (LBG and GG) as additives in the enhancement of startup phase, for UASB reactors treating municipal sewage. A comparative study was taken forward to ascertain their competence and the following conclusions were drawn.

Both the biopolymers were successful in enhancing granulation within a shorter span than the cationic polymer chitosan. Though all these polymers possess the same polysaccharidic structure, the improved performance of LBG and GG can be related to their viscous nature and high surface area. This has aided in fostering more of microbes within the system and thereby enhanced the treatment potential. Of these two polymers LBG excelled as the best one with a maximum COD removal of 92.5% in 40 d. Also the granules formed had a good settleability with an SVI of 26 mL/g – sign of a dense sludge bed getting formed. SMA and Morphological study of granules further confirmed the presence of active methanogens- shorter granulation period had no compromise with the microflora and their activity. Hence the idea of using gum based polymers for accelerated startup of UASB reactors can be considered as a robust technology.

The usage of these bio-polymers is yet to be explored for various high strength industrial wastewaters, which will fetch them a wider application. Also a separate study can be taken up to ascertain the optimum dose of these polymers, to be added. As the viscosity and hydration rate of these polymers are more susceptible to temperature variations, these parameters are to be analyzed in detail.

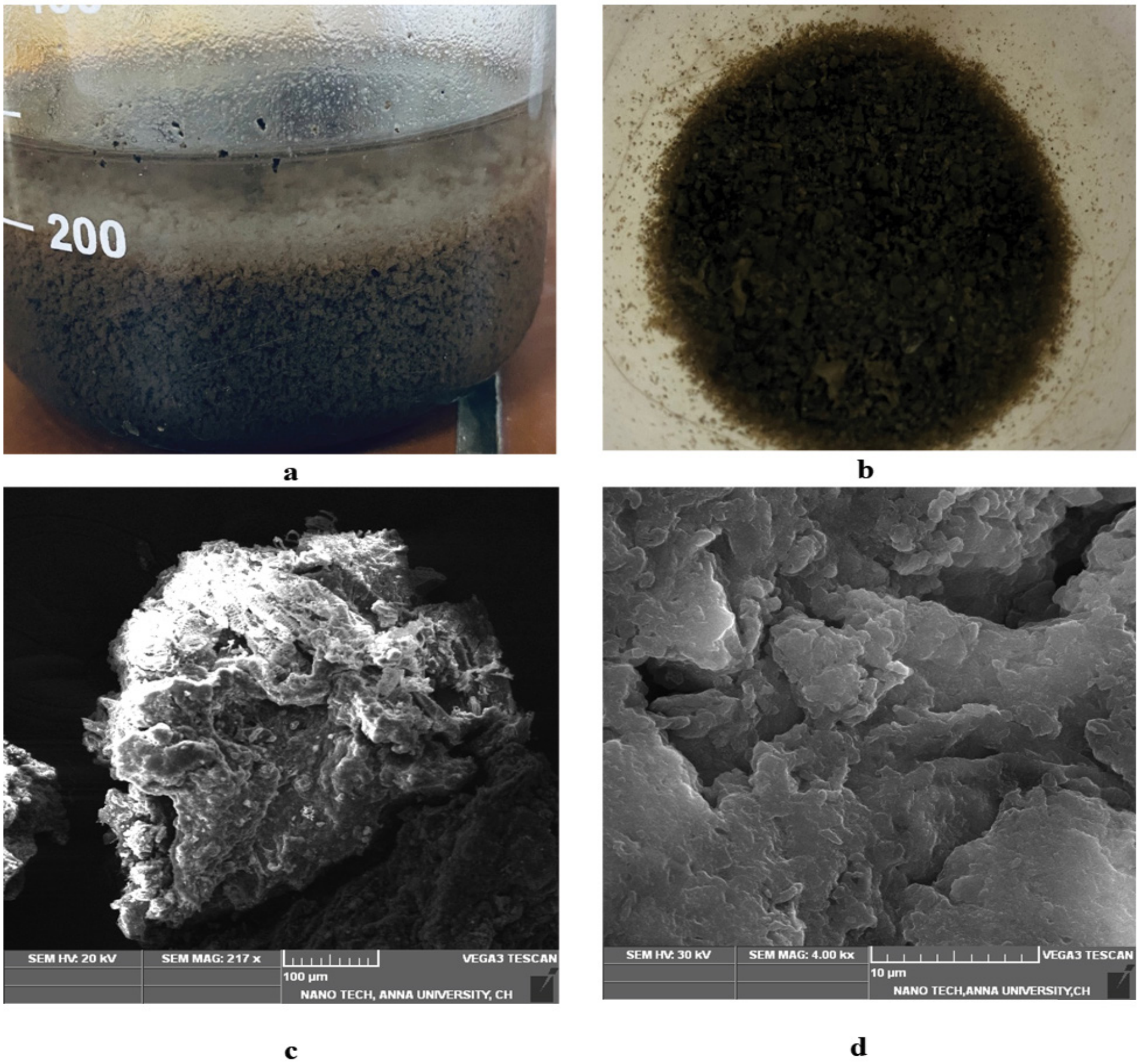


Fig. 9. Morphological study of granules: (a) emergence of granules in LBG reactor on 40th day, (b) visual examination of the segregated granules, (c) SEM observation of the outer surface of granules in LBG, and (d) microbial community dominated by *Methanosarcina* and *Methanothrix*.

## Acknowledgements

The authors acknowledge center for research – Anna University for the financial support of this research work, through Anna Centenary Research fellowship under Grant [CFR/ACRF/19231191147/AR1].

## Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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