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Enhancing Biogas Production and UASB Start-Up by Chitosan Addition

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1. Introduction

Anaerobic digesters have been applied for the treatment of wastewater yielding biogas as a value by-product. The biogas from the treatment plant can be utilized for generating heat and electricity. Anaerobic bacteria form granules through cell self-immobilization which then settle out as floc aggregates. These granules are dense microbial consortia packed with different bacterial species and contain millions of organisms per gram of biomass (Liu & Tay, 2002; Liu et al., 2003; Sheng et al., 2010). Granules in anaerobic digestion are important for enhancing process efficiency by increasing biomass hold-up. An anaerobic digester with higher biomass hold-up will be better in terms of COD removal and biogas production.

Granular sludge is a prominent characteristic of upflow anaerobic sludge blanket (UASB) reactors. This type of reactor has a longitudinal structure with a gas/liquid/solid separator at the top, while microbial granules with high settling velocity are formed in a thick biomass blanket zone at the bottom (Lettinga et al., 1983). The performance of UASB systems depends upon the granulation process. Unfortunately, a long start-up period is required for the development of anaerobic granules in UASB reactors since anaerobes are slow-growing bacteria (Liu & Tay, 2002; Show et al., 2006a). When seed sludge is not granulated, the UASB start-up periods are relatively long and washout of finely dispersed sludge particles is a typical problem (Poh & Chong, 2009).

The UASB start-up period can be shortened by enhancing sludge granulation. The development of well-settleable granular sludge is the key factor for successful UASB operation (Show et al., 2006b). Both synthetic and natural polymers are known to promote particle agglomeration and have been used to enhance the formation of anaerobic granules (El-Mamouni et al., 1998; Show et al., 2006a; Show et al., 2006b). Chitosan is a natural flocculant that has been used for the solid–liquid separation treatment of livestock wastewater (Garcia et al., 2009). Recently, chitosan in the form of freely moving polymeric chains has been found to enhance sludge granulation and shorten the start-up period of UASB systems (El-Mamouni et al., 1998; Lertsittichai et al., 2007; Liu et al., 2002; Thaveesri et al., 1995).

2. Chitosan as flocculants

Chitosan has been largely employed in many areas, such as photography, biotechnology, cosmetics, food processing, biomedical products (artificial skin, wound dressing, contact lens, etc.) and in a system for controlled liberation of medicines (capsules and microcapsules). In addition, chitosan has been used as a flocculant for the removal of metallic and colouring ions from industrial effluents by bonding the micro-floc particles together to form larger, denser flakes that are easier to separate (de Alvarenga et al., 2010; Renault et al., 2009).

Chitosan is a natural polysaccharide whose structure is similar to extracellular polymeric substances (ECP). ECP are widely known to assist anaerobic cell aggregation. Polymeric chains of ECP enhance flocculation by bridging microbial cells to form an initial microbial nucleus which is the first step in microbial granulation. There are many hypotheses to explain adhesion and aggregation processes by ECP. For example, in one hypothesis, ECP production is thought to occur prior to adhesion and the appearance of polymer materials at the initial site of contact between microbial cells is believed to be caused by the migration of polymer molecules onto the cell surface. In another hypothesis, ECP production is thought to occur after adhesion. In this case, it is believed that bacterial adhesion provides a favorable physiological condition for ECP excretion (El-Mamouni et al., 1998; Liu et al., 2002; Show et al., 2006a).

Chitosan is obtained by partial deacetylation of chitin (de Alvarenga et al., 2010). Chitin is a β-(1→4)-linked polymer of 2-acetamido-2-deoxy-d-glucose (N-acetyl-d-glucosamine) which exists in the exoskeletons of insects, crustaceans and the cell walls of fungi and algae. Basically, deacetylation involves the replacement of acetyl groups in the molecular chain of chitin by complete amino groups (NH_2). Chitosan is a mixture of straight-chain copolymers of N-acetyl-D-glucosamine and D-glucosamine of varying degrees of deacetylation (DD), i.e., with varying average numbers of D-glucosamine units per 100 monomers (Khan et al., 2002; Sabnis & Block, 1997). Chitosan also has the advantage that it is naturally biodegradable and therefore should have little adverse affect on human health.

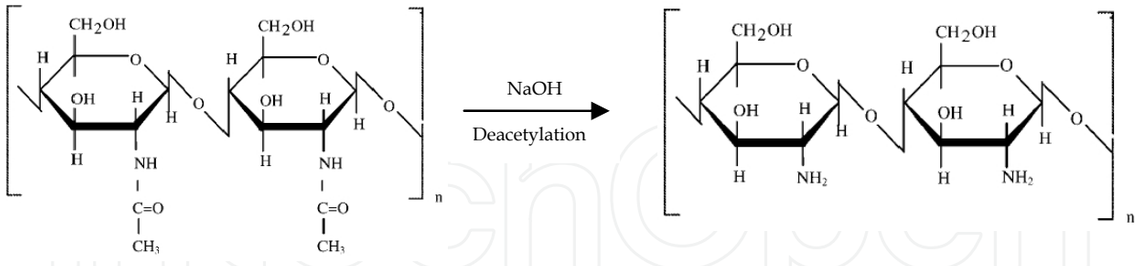


Fig. 1. Deacetylation of chitin to chitosan

Chitosan is insoluble in water, organic solvents and aqueous bases, but it is soluble after stirring in acids such as acetic, nitric, hydrochloric, perchloric and phosphoric acids (de Alvarenga et al., 2010). The glucosamine moieties in chitosan carry free amine groups that are protonated in an acidic environment. The amount and the positions of the glucosamine determine the charge and the charge distribution in the chitosan molecule. Changes in charge density have an effect on the dissolution and binding properties of chitosan (Domard, 1996). The degree of deacetylation also controls the degree of crystallinity and hydrophobicity of chitosan (Vander Lubben et al., 2003). Chitosan enhances the flocculation of sludge, and the flocculation efficiency depends on both DD and molecular weight (MW).

3. The effects of chitosan characteristics and environmental conditions on flocculation of anaerobic sludge

The flocculation efficiency of chitosan is sensitive to its characteristics. The most important characteristics of chitosan for flocculation efficiency are the degree of deacetylation and molecular weight since these are the main factors that affect particle size, particle formation and aggregation in the flocculation process. However, environmental conditions, i.e. pH and ionic strength, are also important in the dissolution and the charge of chitosan for flocculation process.

3.1 Effect of % deacetylation (DD) of chitosan

pH 7 is a typical starting pH in a UASB and most other anaerobic digesters (Lettinga et al., 1980). Kaseamchochoung et al. (2006) investigated the effect of %DD of chitosan on anerobic flocculation by using chitosan with different degrees of deactylation: M85 (DD = 85%) and M70 (DD = 70%) at pH 7. Their experimental procedure was as follows. In the flocculation assay, an initial sludge suspension was transferred into a beaker and a chitosan stock solution was added to achieve a concentration of 0 to 45 mg chitosan/g oven-dried (o.d.) sludge. The suspension was then stirred. The pH of the suspension was adjusted to 5, 6, or 7, with either 1% acetic acid or 3% sodium carbonate, depending on the pH of chitosan added to the suspension. After continuous mixing, the turbidity of supernatant was determined using a turbidimeter. The flocculation was calculated from the decrease in turbidity of supernatant after the treatment with chitosan compared with a reference without chitosan.

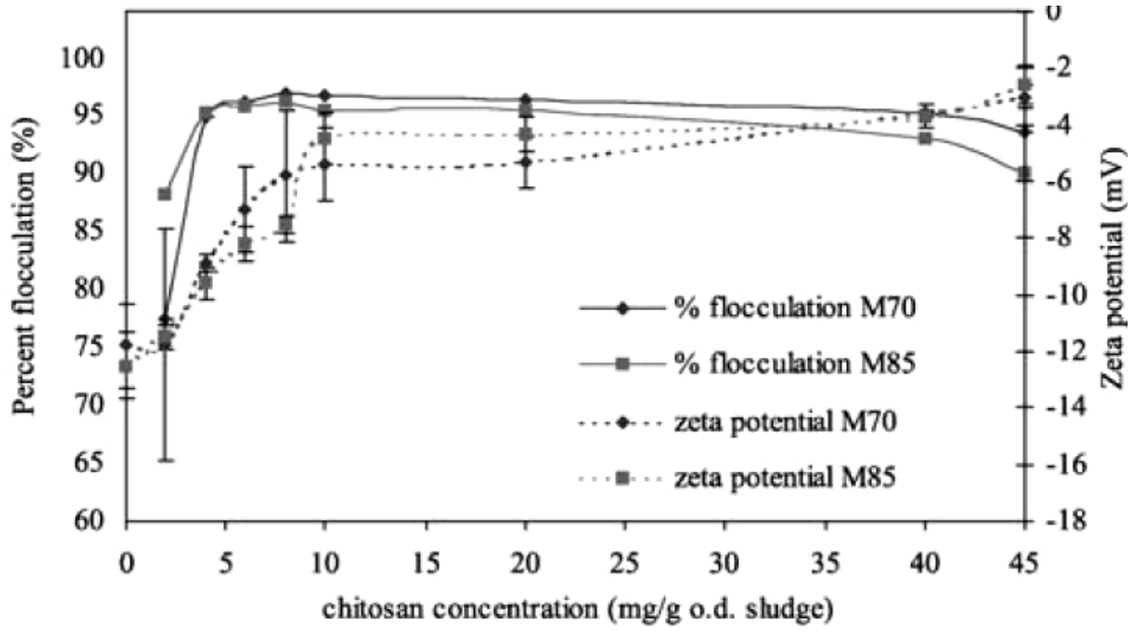


Fig. 2. Flocculation and zeta potential as a function of chitosan concentration in sludge suspension at pH 7 with ionic strength of 0.1 M (from Kasemchochoung et al., 2006. Reprinted with permission from *Water Environment Research*. Volume 78, No. 11, pp. 2211 to 2214, Copyright © 2006 Water Environment Federation, Alexandria, Virginia.)

Kaseamchochoung et al. (2006) found that at a low concentration (2 mg chitosan/g o.d. sludge) chitosan M85 gave approximately 90% flocculation, whereas M70 gave only approximately 80% flocculation (Fig. 2). However, at a concentration of 4 mg chitosan/g o.d. sludge the flocculation efficiencies of M70 and M85 became approximately equal at 95% flocculation and then remained approximately equal up to concentrations of 45 mg chitosan/g o.d. sludge (Fig. 2).

3.2 Effect of chitosan molecular weight

Kaseamchochoung et al. (2006) also studied the effect of molecular weight of chitosan on flocculation. They controlled the deacetylation of chitosan samples at 83 ± 2% and studied two levels of molecular weight ($3.5x10^5$ and $1.4x10^6$ dalton; Da). They found that the low molecular weight chitosan had a higher flocculation efficiency than the high molecular weight chitosan. Following Gregory (1993), they suggested that a possible explanation is that the longer polymers make more surface contacts per molecule and possibly saturate the cell surfaces, leaving no space for other polymers from different cell particles to initiate bridging.

3.3 Effect of environmental pH and ionic strength

Kaseamchochoung et al. (2006) found that the progression of anaerobic digestion in a UASB may cause pH to drop to 6 or even lower. At pH 6 and 7, approximately 90% flocculation was obtained by adding 2 mg chitosan/g o.d. sludge of chitosan M70 and M85. However, at pH 5, approximately 95% flocculation was obtained at the same chitosan concentration (Fig. 3).

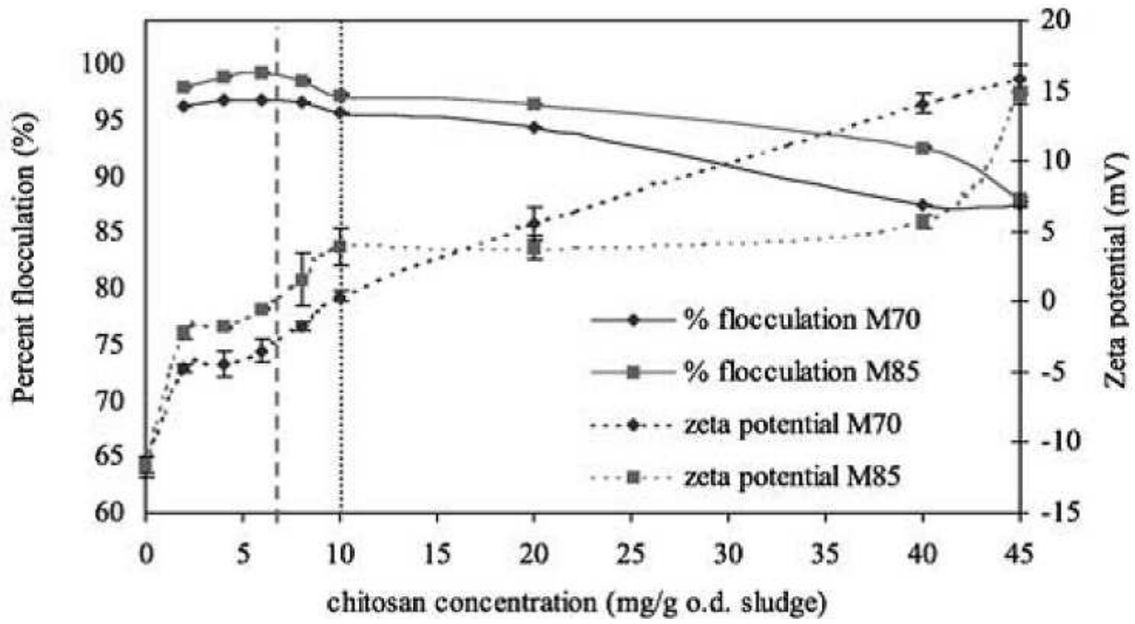


Fig. 3. Flocculation and zeta potential as a function of chitosan concentration in sludge suspension at pH 7 with ionic strength of 0.1 M. Vertical lines indicate the position of the CNP: (.....) for M70 and (_ _ _) for M85 (from Kasemchochoung et al., 2006. Reprinted with permission from *Water Environment Research*. Volume 78, No. 11, pp. 2211 to 2214, Copyright © 2006 Water Environment Federation, Alexandria, Virginia.)

Similar results were obtained by Roussy et al. (2004). They studied chitosan efficiency at three different pH values (pH 5, 6.3, and 9). They found that a lower chitosan dosage (87% DD) was required at pH 5, while a significantly higher dosage of chitosan was required at pH 9 to obtain a residual turbidity below a fixed limit of 5 formalin turbidity units. Their explanation was that two possible mechanisms were possible at pH 5—(a) coagulation by charge neutralization and (b) flocculation by entrapment in the polymer network. However, at pH 9 only the latter mechanism is possible, but its effect can only be significant at a high chitosan concentration.

Kaseamchochoung et al. (2006) found that both chitosan M70 and M85 were able to flocculate anaerobic sludge even when the system pH dropped to 5. A small degree of restabilization was observed after the charge neutralization point (CPN). That is, the percentage of flocculation dropped only slightly after the CPN, whereas zeta potential values became positive. A possible explanation given in Kaseamchochoung et al. (2006) is that the charge density of chitosan is greatly influenced by pH (Strand et al., 2001). Because the intrinsic pKa of chitosan is close to 6.5, most amine groups are protonated at pH 5, but become significantly less protonated when the pH increases. The polymer is therefore more highly positively charged at pH 5 than at pH 7. At pH 7, chitosan with 70%DD contains a lower charge density than chitosan with 85%DD, and the performance of chitosan (70%DD) would be noticeably lower at a low chitosan dosage (Fig. 2). Kaseamchochoung et al. (2006) suggested that charge density may play an important role in the flocculation mechanism and that this is not surprising because electrostatic forces are typically the main cause of polyelectrolyte adsorption on an oppositely charged surface. They concluded that chitosan has the potential to be used as an effective cationic bioflocculant, which is able to function either in acidic or neutral conditions, and that only relatively small amounts of chitosan (less than 4 mg/g dried sludge) are required.

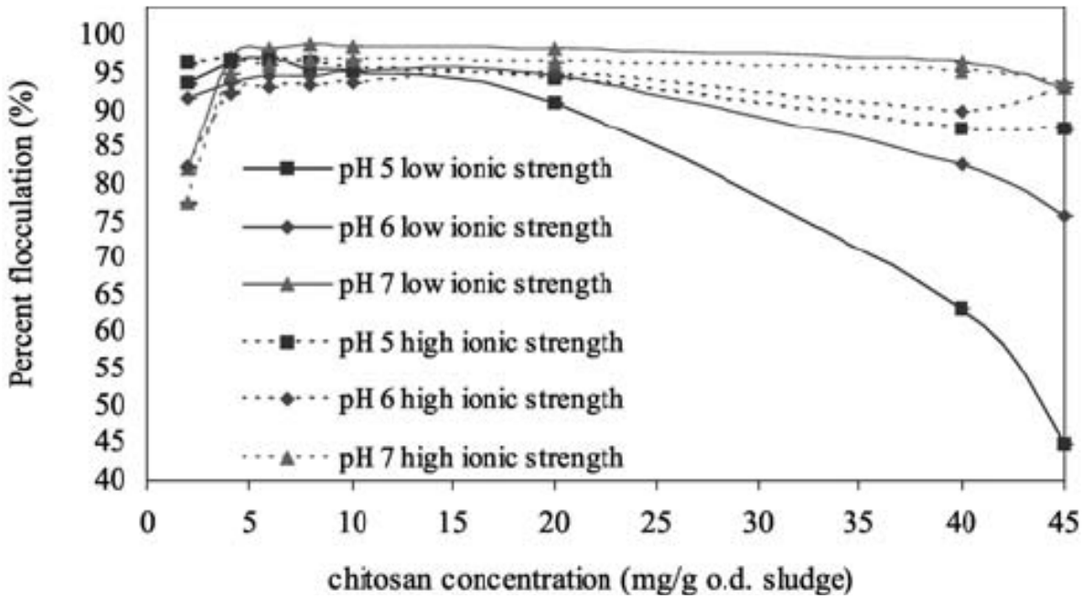


Fig. 4. Percent flocculation as a function of chitosan M70 concentration in sludge suspension at different pH values and ionic strengths (from Kasemchochoung et al., 2006. Reprinted with permission from *Water Environment Research*. Volume 78, No. 11, pp. 2211 to 2214, Copyright © 2006 Water Environment Federation, Alexandria, Virginia.)

In addition to pH, ionic strength of a medium is also a major factor affecting flocculation. Kaseamchochoung et al. (2006) investigated the effect of ionic strength on flocculation by chitosan of high (0.1 M) and low (0.01 M) ionic strength. At pH 7, ionic strength did not signficantly influence the pattern of flocculation by chitosan M70 and the flocculation remained at approximately 95%. In contrast, at pH 5, chitosan M70 performed significantly better in the high-ionic-strength medium. Under the low ionic strength condition, the flocculation dropped from approximately 95% to 45% (Fig. 4). A possible explanation for the effect of salt was obtained from classical theories of colloidal stability (Strand et al., 2001). The extension of the double layer, which causes electrostatic repulsion between charged colloids and the range of repulsion forces, decreases with increasing ionic strength in the surrounding medium. Therefore, bacterial cells should be able to come closer and thus flocculate better in a high ionic strength medium.

4. Effect of chitosan on the performance of UASB treating fruit-processing wastewater

According to Kaseamchochoung et al. (2006), chitosan with 85%DD and MW of $3.5x10^5$ Da yielded the highest flocculation efficiency and versatility to changes in environmental pH and ionic strength.

Lertsittichai et al. (2007) studied the efficiency of chitosan in a UASB reactor treating tropical fruit-processing industry wastewater. The details of their study were as follows. The fruit canning factory wastewater consisted mainly of sugar. The wastewater characteristics were: COD 5,130 to 5,520 mg/L, volatile fatty acid (VFA) 703 to 1,834 mg/L, pH 5 to 6 and ionic strength of 0.028 to 0.036 M.

Two identical UASB reactors with a working volume of 30 L were employed for the comparative study. The startup period was operated at a hydraulic retention time (HRT) of 85 hours, corresponding to an organic loading rate (OLR) of 1.45 g COD/L·d. Chitosan at a concentration of 2 mg/g suspended solids was added to the reactor on the second day and the same amount was added on the 37^{th} operating day. The HRT of both reactors were reduced in a stepwise fashion, at 85, 65, 45, and 35 hours, when the COD removal was higher than 80% for at least 3 times the HRT.

Throughout the operation of the process, the OLR values ranged from approximately 1 to 4 g COD/L·d. Lertsittichai et al. (2007) found that the UASB with chitosan addition gave 9 to 59% lower COD effluent and had a 4 to 10% higher removal efficiency than the control UASB. The low VFA values corresponded to high biogas production because VFA is an intermediate for methane production. The UASB with chitosan addition gave a lower VFA value and a 35% higher biogas production rate than the control (Fig. 5).

Effluent VSS refers to biomass washout. Lertsittichai et al. (2007) found that the biomass washout increased during the initial operation period of both reactors. After 35 days, the biomass washout decreased due to granule formation. The biomass washout from the UASB with chitosan addition was 16 to 68% lower than that from the control. The UASB with chitosan addition was found to consistently have 24 to 37% higher average particle sizes than the control, corresponding to the lower biomass washout.

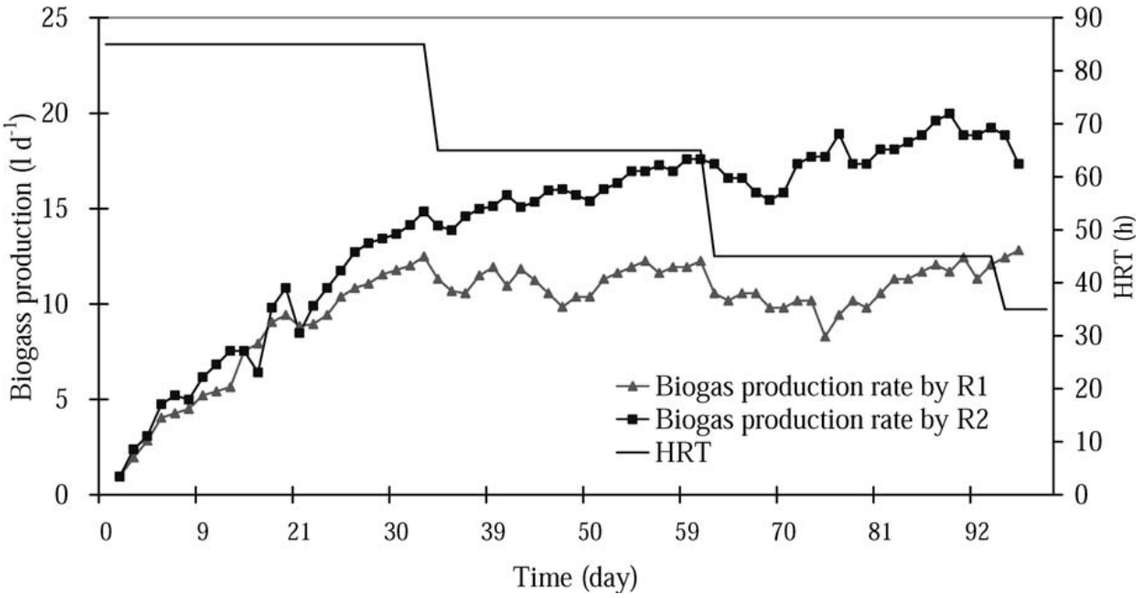


Fig. 5. Biogas production against time (from Lertsittichai et al., 2007). R1 is the control UASB reactor and R2 is the reactor with chitosan addition. Reprinted with permission from *Water Environment Research*. Volume 79, No. 7, pp. 802 to 806, Copyright © 2007 Water Environment Federation, Alexandria, Virginia.

In addition, Lertsittichai et al. (2007) found that the UASB with chitosan addition consistently had a 6 to 41% longer solids retention time (SRT) than the control corresponding to a lower effluent VSS and a higher average particle size. The VSS from the bottom sampling ports of the UASB with chitosan addition was higher than that of control, leading to greater overall sludge density. From their observations, Lertsittichai et al. (2007) concluded that chitosan helped sludge pellet development. They gave the possible explanation that the cell surfaces of bacteria carry negative charges, and the electrostatic interactions between them are repulsive. Therefore, a cationic polymer, such as chitosan, assists the flocculation of the bacteria leading to faster sludge formation and a higher density of sludge retained in the reactor.

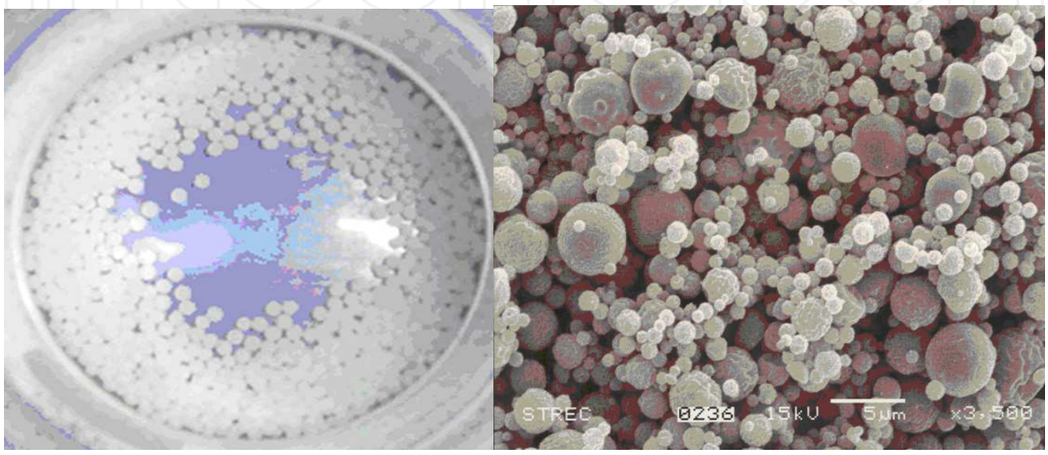
Overall, Lertsittichai et al. (2007) used only small amounts of chitosan (two injections with 2 mg chitosan/g suspended solids at each injection). They saw no sign of inhibition to biomass activity. Throughout the course of their experiment at a mesophilic temperature (35^{o}C), the UASB with chitosan addition clearly showed superior performance to the reactor without chitosan, with 9 to 59% lower effluent COD, 4 to 10% higher COD removal, up to 35% higher biogas production rate, and decreased washout of biomass and increased granular size.

5. Investigation of chitosan in different forms

Chitosan is available commercially in three forms: solution, flake and powder. The prices of chitosan in the forms of solution, flake and powder range between 50 to 70 baht/L, 700 to 900 baht/kg and 750 to 2,300 baht/kg, respectively. Chitosan in the form of freely moving polymeric chains has previously been found to enhance sludge granulation and shorten the

start-up period of UASB systems (El-Mamouni et al., 1998; Lertsittichai et al., 2007; Liu et al., 2002; Thaveesri et al., 1995).

The effectiveness in enhancing granulation of different forms of chitosan, i.e. solution, bead and powder, has also been studied by Nuntakumjorn et al. (2008). They prepared chitosan solution by dissolving chitosan in acetic acid solution (1% w/v). In preparing chitosan powders, they used a spray dryer to spray-dry chitosan solution (1% w/v). In preparing the chitosan beads, they dropped the chitosan solution (4% w/v) into a solution of KOH and ethanol. The chitosan beads were found to have spherical shape, white color and looked like glutinous pellets. The appearance of the chitosan beads is shown in Fig. 6.



(a) (b)

Fig. 6. (a) Chitosan beads in the KOH/Ethanol solution (b) SEM micrograph with 3500x of chitosan powders (from Nuntakumjorn et al., 2008)

Nuntakumjorn et al. (2008) used two identical reactors, with a working volume of 5.3 L, running in parallel. A sludge suspension with an initial VSS concentration of 12 g VSS/L was inoculated into the reactors. The acclimation of the sludge was carried out until the COD removal was approximately 80%. The reactors were run with a HRT of 1.5 day corresponding to an OLR of 1.45 g COD/L·d. Chitosan in the different forms was introduced into the reactors on the second operating day of the start-up period at dose rates of 2 mg chitosan/g suspended solids.

A summary of the results of Nuntakumjorn et al. (2008) is as follows. When comparing between the UASB with no chitosan addition and the UASB with chitosan addition in the solution form, the UASB with chitosan addition was found to have a 9 to 59% lower effluent COD, 5 to 7% higher COD removal, up to 25% higher biogas production rate, 21 to 39% lower biomass washout, 37% larger particle size and 4 day longer sludge retention time.

When comparing between the UASB with chitosan addition in the solution form and with addition in the bead form, the UASB with chitosan solution was found to have 5 to 17% lower effluent COD, 16 to 45% higher COD removal, 7 to 20% lower biomass washout and 3 to 17% higher biogas production than the UASB with chitosan beads. The reduced effectiveness of chitosan in the bead form might be caused by a lower amount of chitosan in the bead form and by insufficient contact between the chitosan beads and biomass.

When comparing between the UASB with no chitosan addition and the UASB with chitosan addition in the powder form, no differences were found in terms of COD removal, biogas production and biomass washout. The average COD removal of the UASB with chitosan addition was approximately 80% and that without chitosan was approximately 81%. The biogas production rate was 9.85 L/d and 10.23 L/d for the UASB with and without chitosan addition, respectively. Both UASB reactors had biomass washout in the range of 0.6 to 1.5 g VSS/L. Although chitosan powders have net positive charge, the electrostatic interaction between the negatively charged bacteria was not significantly reduced. Nuntakumjorn et al. (2008) concluded that chitosan powders does not enhance the granulation process and UASB performance.

6. Effect of chitosan on microbial diversity in UASB treating POME under thermophilic condition

Palm oil mill effluent (POME) contains high COD and biochemical oxygen demand (BOD). POME consists of a wide range of biological substances from complex biopolymers such as proteins, starches and hemicelluloses to simple sugars and amino acids. POME may also contain dissolved oil and fatty acids, glycerin, crude oil solids and short fibers as well as soluble materials that are harmful to the environment. Since POME is discharged at high temperatures (80–90^{o}C), both mesophilic and thermophilic temperatures have been widely applied for POME treatment by anaerobic digestion.

6.1 Effect of chitosan on UASB treating POME

It has been reported that thermophilic operation of anaerobic reactors provides some advantages over mesophilic operation in areas such as higher rates of substrate degradation and biogas production. However, mesophilic reactors can be preferable because of greater process stability (Mustapha et al., 2003; Poh & Chong, 2009). Operating temperature is a major factor that greatly influences digester performance (Choorit & Wisarnwan, 2007; Poh & Chong, 2009; Yu et al., 2002).

The effects of chitosan as a sludge granulation accelerator during the transition from mesophilic (37^{o}C) to thermophilic condition (57^{o}C) has been investigated by Khemkhao et al. (2011). They used two UASB reactors, with a working volume 5.3 L, both of which they inoculated with mesophilic anaerobic sludge. The sludge was then acclimatized to a thermophilic condition with a stepwise temperature increase of 5^{o}C from 37 to 57^{o}C. The OLR ranged from approximately 2 to 9.5 g COD/L·d. One of the reactors was then injected with a chitosan dosage of 2 mg chitosan g/VSS on the first day of operation and the second reactor was used as a control.

At all times during the operation of the two reactors, the UASB with chitosan addition was found to have 5% higher COD removal efficiency and 16 L/d higher biogas production rate (7.82 L/g VSS removed·d) than that of the control. The methane contents of both reactors were found to be similar, with approximately 78% methane content for UASB with chitosan addition and 76% for the control. The effluent VSS in both reactors was found to increase with increase of OLR. The UASB with chitosan addition was found to have 6 to 23% lower effluent VSS than that of the control. Khemkhao et al. (2011) concluded that the UASB with chitosan addition had consistently better performance than the control.

6.2 Effect of chitosan on microbial diversity in UASB treating POME

The mechanism of anaerobic digestion in methane production consists of a series of complex metabolic interactions between various types of microorganisms in the absence of oxygen. Anaerobic digestion is mediated through processes of hydrolysis, acidogenesis, acetogenesis and methanogenesis. Khemkhao et al. (2011) used 16S rRNA targeted denaturing gradient gel electrophoresis (DGGE) fingerprints to study the microbial communities during anaerobic digestion. They found that bacteria and methanogens could both be detected in the UASB reactors operating both with and without chitosan addition.

6.2.1 Effect of chitosan on bacterial diversity in UASB treating POME

In their experiments, Khemkhao et al. (2011) found that DGGE patterns of bacterial diversity of the three bacterial groups, hydrolytic, acidogenic and acetogenic, persisted at all operating temperatures. However, the distribution of their members among bacteria in each group did show small changes under the different operating conditions. By the end of the operating period, the UASB with chitosan addition was found to contain a lower proportion of hydrolytic bacteria and a higher proportion of acidogenic bacteria than the control. However, the diversity of acetogenic bacteria was found to be similar in the two reactors. Sulfate-reducing bacteria were detected in the control but not in the chitosan reactor.

It is known (Bitton, 1994) that hydrolytic, acidogenic and acetogenic bacteria work together to degrade complex organic matters into acetate, CO_2 and H_2. Hydrolytic bacteria begin the process of degradation by breaking down complex organic molecules such as proteins, cellulose, lignin and lipids into soluble monomer molecules by extracellular enzymes, i.e., proteases, cellulases and lipases. The monomer molecules produced are amino acids, glucose, fatty acids and glycerol. These monomers are then degraded by the acidogenic (acid-forming) group of bacteria which convert them into organic acids, alcohols and ketones, acetate, CO_2, and H_2. The organic acids produced include acetic, propionic, formic, lactic, butyric, and succinic acids. The alcohols and ketones produced are ethanol, methanol, glycerol and acetone. In the final stage, the acetogenic bacteria (acetate and H_2-producing bacteria) convert the fatty acids, alcohols and ketones into acetate, CO_2 and H_2.

6.2.2 Effect of chitosan on archaeal diversity in UASB treating POME

Khemkhao et al. (2011) found that all methanogen DGGE bands observed in the control were also detected in UASB with chitosan addition. The observed acetotrophic methanogens were the family *Methanosarcinaceae* and the species *Methanosaeta soehngenii*. The observed hydrogenotrophic methanogens were the order *Methanomicrobiales*, the genus *Methanolinea* sp. and the species *Methanoculleus marisnigri*. On the other hand, some of the acetotrophic methanogens were observed in the UASB with chitosan addition, but not in the control. These acetotrophic methanogens were the order *Methanosarcinales,* the species *Methanosaeta thermophila* and *Methanosaeta harundinacea*.

Methanogenic archaea are oxygen–sensitive anaerobes (Bitton, 1994). They can grow into individual cells, filamentous chains, cubes and/or sarcina. Methanogens are subdivided into two subcategories: (i) hydrogenotrophic methanogens and (ii) acetotrophic methanogens.

Hydrogenotrophic methanogens convert H_2 and CO_2 into CH_4. Acetotrophic methanogens convert acetate into CH_4 and CO_2. The acetotrophic methanogens grow slower than the acid-forming bacteria. About two-thirds of CH_4 is derived from acetate conversion by acetotrophic methanogens. The other third is the result of H_2 and CO_2 reduction by hydrogenotrophic methanogens.

As stated above (Khemkhao et al., 2011), lower biomass washout was observed from the UASB with chitosan addition than from the control, especially at higher biogas production rates. The DGGE analysis shows that UASB with chitosan addition contains higher populations of *Methanosaeta* species than the control. It can be concluded that the chitosan helped to retain these methanogens, thus resulting in higher populations of acetotrophic methanogens.

Tiwari et al. (2005) and Tiwari et al. (2006) have reported that acetotrophic methanogens significantly accelerate granule development. Higher population of acetotrophic methanogens may in turn lead to higher methane production in the reactors with chitosan addition.

Chitosan has been reported to act like an ECP in enhancing the aggregation of acidogens. As shown in Fig. 7, the aggregated acidogens then form granules with highly elastic outer

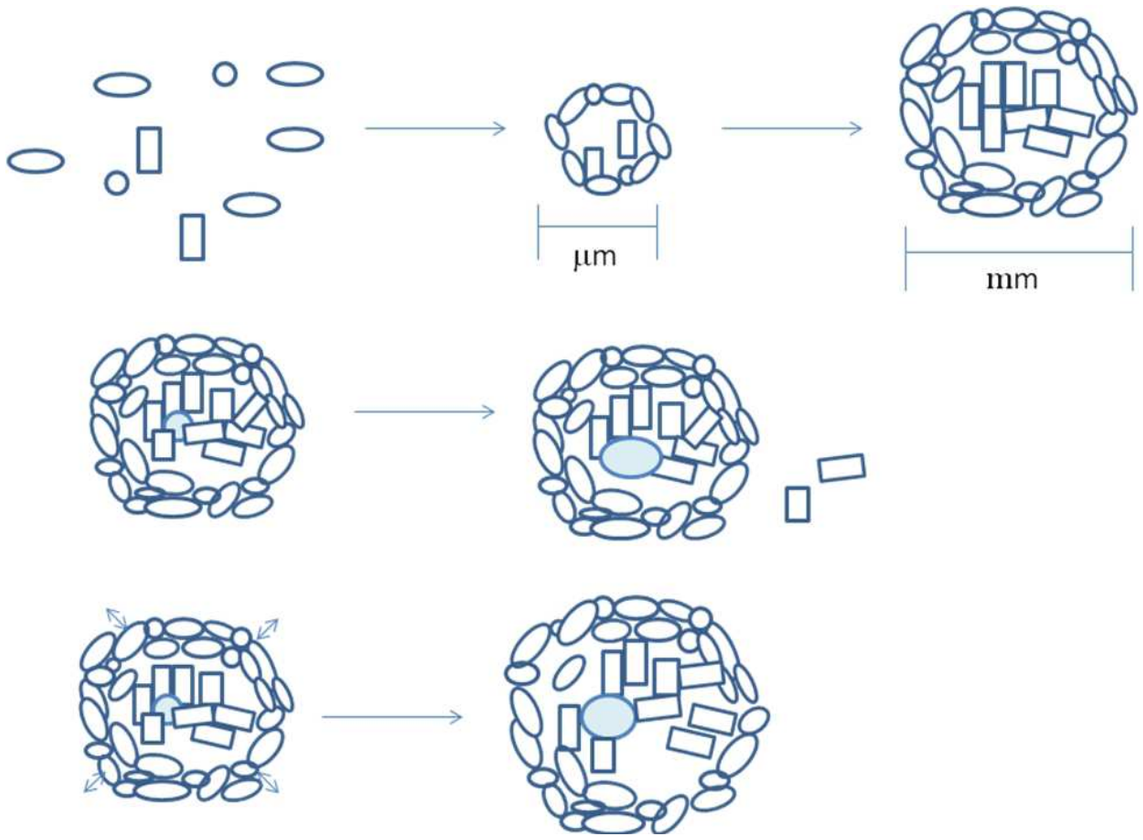


Fig. 7. Scheme of granule formation. Top: Surface tension model according to Thaveesri et al. (1995) and Hulshoff Pol et al. (2004). Middle: Some circumstances in the control reactor. Bottom: Enhanced aggregation by chitosan in UASB with chitosan addition (from Khemkhao et al., 2011)

hydrophilic layers around a core of methanogens. According to Hulshoff Pol et al. (2004) and Thaveesri et al. (1995), the acidogens (round and rod cells) aggregate by forming ECP. Dispersed cells are washed out, while some methanogens (rectangular cells) are enclosed inside, becoming the nucleus of a granule with an outer elastic hydrophilic layer formed by ECP-rich acidogens and an inner core of hydrophobic methanogens. Chitosan has been thought to act like ECP in aggregating anaerobic sludge (El-Mamouni et al., 1998). Therefore it may increase the elasticity of outer hydrophilic layers of the granular samples. In UASB with chitosan addition, the growing methanogens are better protected inside an acidogenic layer and may become less susceptible to adhesion to gas bubbles (filled circles) and consequently may be less washed out from the reactor than those in the control.

The polymer additives appear to play a similar role to naturally secreted ECP in aggregating anaerobic sludge. The addition of polymers to anaerobic systems changes the surface properties of bacteria to promote association of individual cells. Polymer may form a solid and stable three-dimensional matrix within which bacteria multiply and daughter cells are then confined (Liu et al., 2002; Show et al., 2006a; Uyanik et al., 2002).

In addition, Show et al. (2006b) have reported that adding an appropriate dosage of polymer in the seeding stage accelerates the start-up time by approximately 50% and the granule formation by approximately 30%. In addition, granules developed in polymer-assisted reactors exhibited better settleability, strength and methanogenic activity at all OLRs tested. Positively charged polymer forms bridges among the negatively charged bacterial cells through electrostatic charge attraction. The bridging effect would enable greater interaction between biosolids resulting in preferential development and enhancement of biogranulation in UASB reactors (Show et al., 2006a).

In the experiments of Khemkhao et al. (2011), the UASB reactor with chitosan addition was treated with a one-time chitosan dose of 2 mg chitosan/g VSS on the first operating day. The performance of the UASB reactor may be further enhanced by more injections of the chitosan solution. However, the evidence from the one-time chitosan dose of 2 mg chitosan/g VSS on the first operating day was that the initial stage of granulation was very important for forming high quality granules.

7. Conclusion

Chitosan is a biopolymer which can be used to enhance the sludge granulation process and UASB performance. Flocculation efficiency of chitosan was sensitive to its characteristics as well as to the pH and ionic strength of the environment. An increase in the deacetylation of the chitosan from 70 to 85% led to a two-fold reduction in the chitosan concentration necessary to achieve 90% flocculation at pH 7 (Kaseamchochoung et al., 2006).

Chitosan, with a degree of deacetylation of 85% and molecular weight of 3.48×10^5 Da, yielding high flocculation efficiency (85 to 100% flocculation) and broad flocculation region (2 to 45 mg/g suspended solids), was shown to accelerate granulation in a 30-L pilot-scale UASB used to treat wastewater from a tropical fruit-processing industry (Lertsittichai et al., 2007).

For the same amount of chitosan, chitosan in the solution form was shown to be significantly better at enhancing the granulation process and the UASB performance than chitosan in bead or powder forms (Nuntakumjorn et al., 2008).

For POME treatment, the biogas production rate and the COD removal of the UASB with chitosan addition was on an average 16% and 5%, respectively, higher than that of the control. A DGGE analysis indicates that the chitosan helped to retain the methanogens in the genus *Methanosaeta*, thus resulting in higher populations of acetotrophic methanogens. Further investigations are required to determine optimal chitosan dosages and the optimal times to add chitosan under thermophilic conditions (Khemkhao et al., 2011).

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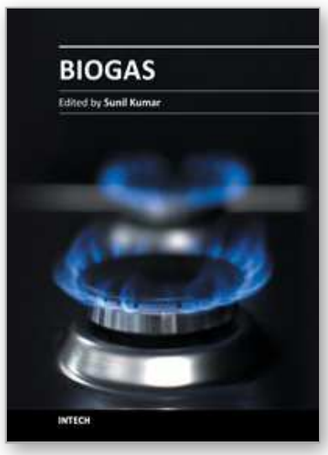
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