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A review on granules initiation and development inside UASB Reactor and the main factors affecting granules formation process

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Abstract
Decades of investigations and explorations in the field of anaerobic wastewater treatment have resulted in significant indications about the role importance of sludge granules in biodegradation anaerobic process. It is believed that the development of anaerobic granules is reflecting an important role on the performance of reactor. An overview on the concept of up-flow anaerobic sludge bed UASB reactor operation as well as the main parts that reactor consists of is briefly explained in this paper, whereas the major theories of anaerobic granules formation are listed by related researchers. The correlations and compositions of such sludge granule have been specifically explained. It is believed that the extracellular polymer (ECP) is totally responsible of bacterial cell correlations and the formation of bacterial communities in the form of granules. In addition, the dependable factors for the performance of anaerobic granules formation process e.g. temperature, organic loading rate, pH, and alkalinity, nutrients, and cations and heavy metals have been discussed in this paper. Strong evidences proved that the process of gas production in the form of biogas is related to the methanogens activities, which are practically found in the core of granules. The aim of this review is to explore and assess the mechanisms of granules initiation and development inside UASB reactor.

Keywords: UASB reactor, Anaerobic granulation, ECP, Microorganisms, Biogas.

1. Introduction
Anaerobic wastewater treatment has become the most attractive method due to its low production of biomass, low cost effectiveness and low energy demand [**1**]. In general, it has completely been believed that anaerobic method of treatment is more economic than other kind of treatment such aerobic wastewater treatment. Particularly, up-flow anaerobic sludge bed (UASB) reactor can be considered the most popular system of secondary treatment due to its several advantages comparing to other systems as conventional anaerobic digesters, considering that UASB reactor has the gas collection as additional part on which it will reduce the effect of releasing gas emissions into atmosphere as it occurs in conventional ponds [**2**]. UASB reactor has probably designed to treat high concentration of wastewater [**3**]. Various researches have widely been conducted in the field of wastewater treatment within employing UASB reactor [**4**,**5**].
The first observation of granules was reported by Young and McCarty 1969 in their experiment of anaerobic filter system [**6**]. However, sludge granules had developed quite slowly due to the difficulty in understanding the process and the insufficient funds at that time. The successful treatment in UASB

reactor is principally attributed to the formation of anaerobic granular in sludge bed [**7**], where by the microbial communities are playing very important roles on digesting the substrates to biogas. The granulation and the microbial community is a complex science and it is very hard to explain all its specification. This item of the study is mentioned the fundamental process of granulation in UASB reactor. Various theories have explained the role and the behaviours of microbial communities inside UASB reactor. However most of the theories have indicated that acetotrophic methanogen Methanosaeta plays a key role in granulation growing. On other hand, some of them have been believed that Methanosarcina aggregations had enhanced the granule formation [**7**]. The granulation theories are in agreement with the sludge granules initiation considering that bacterial adhesion is the initial stage which can be described as (a physical-chemical process) [**8**]. The growth process of the particles required stabilizing operation, avoiding the particles washouts, which are mostly assessed to be as the main concern of granules growing. However, granulation development is totally depending on the effect of pH and temperature [**7**]. However, the mechanisms of granulation formation are still not sufficiently clear. This paper will review the main concept of granules growth in UASB reactor and the most significant attempts have previously done by the researchers will be discussed.

2. UASB bioreactor

The UASB bioreactor concept based on the up-flow feeding of wastewater through the sludge bed at the bottom of reactor. Gas-liquid-solid (GLS) separator is equipped at the top of reactor to separate the solution contents i.e. the liquid as treated effluent, the solids particle is trapped and returned back downward, and the gas is collected from the top of reactor in the form of biogas. The schematic diagram of reactor is illustrated in Figure 1, also all the parts have been clearly shown on the drawing. On the other hand, the successful operation of UASB reactor requires good contact between the substrate of influent and the biomass in sludge bed. Whereby the start-up period of operation includes the microbial communities immobilization through continuous feeding of wastewater into seeded sludge to be developed to granules aggregations which are considered as the key success of the process [**9**]. Sludge granules can be successfully developed inside the reactor by providing appropriate environmental and operational conditions. Practically, the development of granules can be recognized by its particle size that can be measured via particle size analyzer. In subsequent phases of sludge granules development, granule is becoming too complicated in composition. However the initiation of granules are normally started as sludge seeds coping with a reactor status and shocks to be developed as activated granules with microbes, whereby the interval of development is called the start-up period. Subsequently granules will acclimatize to digest the organic components in wastewater to be treated.

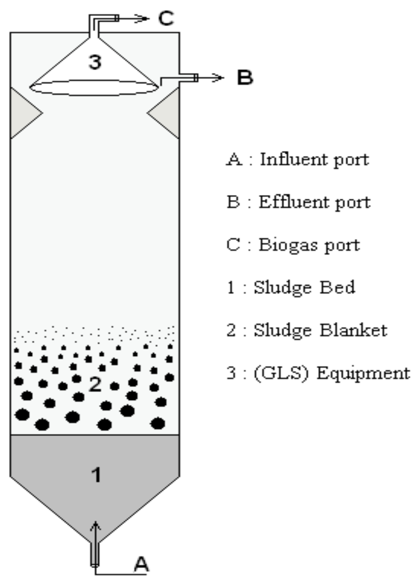

Figure 1. Schematic diagram of UASB reactor

3. Anaerobic granulation theories

The granulation genesis has extensively been classified by researchers into several categories according to precision scientific theories. Liu, 2003 has based onto his classification on the factors or forces that believed to be the reason of granulation phenomenon. Whereby, Liu has classified granules initiation into two main branches: physicochemical model and structure model [**10**]. On the other hand, Hulshoff, 2004 was reported a new classification of granules formation. Table 1 has shown the fundamental basics of theories, which are justified the granules formation process.

Table 1. Overview of the various theories on anaerobic sludge granulation **[**7**]**

Approach		References	Name of theory
Physical		Hulshoff et al.(2004) [**7**]	Selection pressure
		Pereboom, 1994 [**11**]	Growth of colonized suspended solids
Microbial	Physiological	Dolfing, 1987 [**12**]	-
		Sam-Soon et al. 1987 [**13**]	Cape Town hypothesis
	Growth	Wiegant,1987 [**14**]	Spaghetti theory
		Chen and Lun, 1993 [**15**]	-
	Ecological	Dubourgier et al. 1987 [**16**]	Bridging of microflocs
		Morgan et al. 1991 [**17**]	Bundles of methanothrix
		De Zeeuw, 1980	Three types of VFA degrading granules
		McLeod et al. 1990 [**18**]	-
		Vanderhaegen et al. 1992 [**19**]	-
		Ahn, 2000[**20**]	
		Wu et al.(1996) [**21**]	Anaerobic granulation with defined species
Thermodynamic		Zhu et al.(1997) [**22**]	Crystallized nuclei formation
		Thaveesri et al. 1995 [**23**]	Surface tension model
		Schmidt and Ahring, 1996 [**9**]	-
		Tay et al. 2000 [**24**]	Proton translocation–dehydration

4. Seed sludge toward granulation

Basically, and depending on the researchers experiments also, seed sludge that is used for the UASB reactor start-up period to be upgraded to granules, can be provided from any source containing appropriate bacterial flora (Figure 2). Normally, the seed sludge has to be obtained from anaerobic ponds

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sediments, fresh water sediments, septic tank sludge, manure, digested sewage sludge and anaerobic treatment plants [**25**]. The aerobic activated sludge can be used as seed sludge according to the high content of methanogens where it has estimated by Zeikus, 1979 that each gram of suspended solid contained 10^8 methanogens, while the digested sludge contained 2.5×10^{10} methanogens/g [**26**]. Various kind of seed sludge have been successfully used for UASB start-up, in addition of the mentioned above, activated sludge was also used as seed sludge which is giving a better performance as well as a shorter start-up period can be obtained. An investigation has been done by to enhance the granulation process by using various kind of precursors, whereby it has been reported that each syntroph-enriched-methanogenic consortia, Methanothix-enriched and Methanosarcina-enriched nuclei, has a positive role on the acceleration of granules formation, whereas using acidogenic flocs was delaying the granules growth [**27**]. Cations and minerals was essential for the starting phase to granulation, where strong evidences for improvement that are reported in subsequence section of this paper. However, more specifics have to be established to set limited species with seed sludge as a major fraction for anaerobic granulation process.

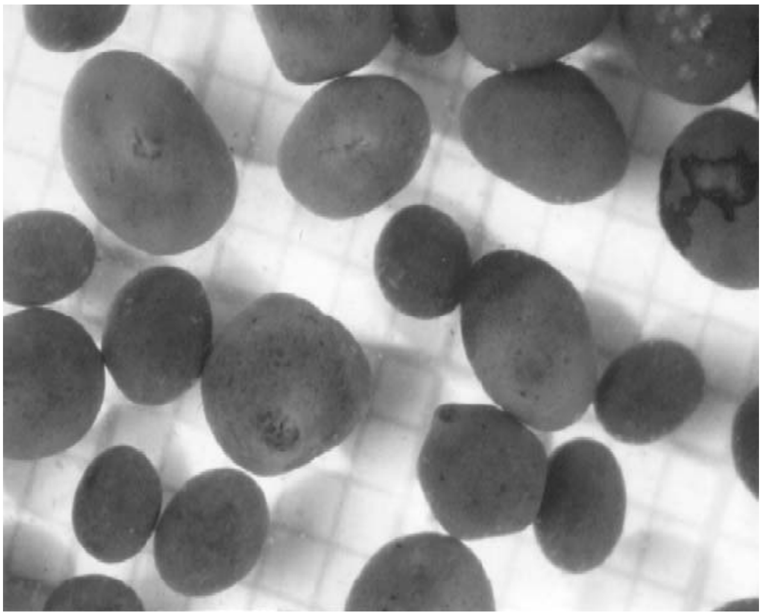

Figure 2. Anaerobic sludge granules from a UASB reactor Hulshoff [7]

5. Granules composition

Each granule has various structural compositions, which is varied according to the wastewater characteristics variation as well as operational condition variation. Granules composition can involve inorganic components, microorganism cells, and extracellular polymer in various rates.

5.1 Inorganic materials

The composition of granules varies due to substrate characteristics, seed sludge source, reactor operational conditions, chemical interactions occurrence, and extrinsic factors. In general, inorganic components are consisted of minerals and ash [**28**]. Where it was reported that the inorganic components in granules can range from 10 to 90% thus it can depend on the wastewater contents, and the operational conditions [**29**]. Beside this, the inorganic contents can vary in the same granules from place to another as much as in the same reactor, and this variation in distribution may normally occur. In fact, under the mesophilic condition of operation, the granules will grow on complex wastewater where it is reported in such case that the ash will be found in low proportion, whereas it can be found in high proportion in case of simple wastewater such as acetate, propionate, or butyrate [**30**]. Reasonably, a relationship between ash and density was indicated that an increase of density is mainly attributed by ash increase in granules [**31**]. Returning to the contents of ash, where it was found that calcium, potassium and iron are involved in ash. In addition of that, the black color of granules is attributed to the existing of FeS in proportion of 30% of the ash content [**32**]. So far, no positive effect of ash on the strength of granules structure has been observed [**26**].

5.2 Microorganism cells

The initiation of granules starts from the microorganisms' adhesion that forming aggregations of bacterial cells that is surrounding by extracellular polymer (ECP) and other components in spherical shapes. Whereby, various theories of granules formation have been lately suggested [**7**]. It is believed that filaments of methanosaeta species are responsible for granulation phenomenon. Whereby, these can purpose as nucleation center for further advance of aggregate [**18**]. Other opinions had believed that through granules initiation, Methanosaeta spp. inhabit the fundamental cavities of Methanosarcina clusters. This investigation has been carried out to young granules with a core of Methanosarcina [**26**]. The first formation of bacterial clusters suggested being acetate. Subsequently, the initial bacterial will create Methanosarcina species or Methanosaeta species [**10**].

5.3 Extracellular polymer (ECP)

The generation of ECP is normally considered as natural property of microbial communities in appropriate ambience and always suggests itself in bacteria, yeast, algae and fungi [**33**].Whereby ECP are formed as a result of microbial cells excretion and corruption as well as organic materials debris [**34**]. Gehr and Henry, 1983 reported that two kinds of ECP are clearly recognized: capsular and slime. Whereby, capsular is always attached the microbial cell, as slime remains without bound to microbial cell. In addition the bulky role of capsular material on the aggregation phenomenon has identified [**35**]. The good understanding of ECP concept of generation and recognize their characteristics are more than essential for the development of biotechnology science for all over the world.

6. Granules size

Strong evidences indicated that the addition of crushed granular methanogenic sludge to digested sewage in an UASB reactor fed with acetate plus propionate may give rise to the development of methanogenic sludge granules with a diameter of 1–4 mm [**36**]. The increase rate of granule size was 31 μm/day for syntroph- seeded granules, 21 μm/day for Methanothix-seeded granules, 18 μm/day for Methanosarcina-seeded granules and only 7 μm/day for acidogenic flocs-seeded granules [**27**]. As mentioned before, it can be concluded that the growth rates in syntroph as well as methanothix seeded sludge were more rapidly than in Methanosarcina and acidogenic flocs seeded granules. On these, the relative variation in granules size from condition to another can be understood.

7. Factors affecting the granulation process

7.1 Temperature

The methanogens are relatively more affected by temperature than acidogens [**37**]. However, many kinds of microorganisms are more acclimatized in mesophilic temperature range. It is reported by (Henze, 1983; Zinder, 1990) that in mesophilic reactors, the optimum range for microorganisms growth is between (30-40)°C, and in relation to the thermophilic temperature, they classified the ranges according to the microorganisms species suitability, whereby for Methanosarcina sp. the optimum temperature for growth is (55-58)°C, Methanosaeta species ~70°C, Methanobacterium species 65–70 °C, and acetateutilizing mixed culture 60–65 °C [**38**,**39**]. However, rising the temperature of reactor may result in prosperity of some species of microorganisms and disintegration, of the other in versus. In fact, mesophilic granules has more sensitivity against temperature shocks than thermophilic granules, whereby any shock in temperature may disintegrate mesophilic granules [**40**]. Nonetheless, it is reported that mesophilic condition for seed sludge is more active and taking less time for start-up operation period than thermophilic condition [**41**]. While much gab in temperature effect of granulation process and development are existing and also strong scientific justifications for the deference of granules structure in each mesophilic and thermophilic are still unclear.

7.2 Organic loading rate

Organic loading rate (OLR) can be considered as the most critical factor that should be carefully adjusted, it is easily to control that factor via COD influent adjustment or adjusting the inflow rate [**42**]. Increase the organic loading rate up to reasonable limit is totally resulted in volatile fatty acids accumulation where the latest will decrease the pH of reactor [**43**]. On the other hand, the disadvantages of OLR decrease are mainly represented by its negative role of disintegrating big granules as a result of lacking in food, where an organic loading rate of (≤1.5) kg COD/m^3.d is not recommended and resulted in granules degeneration [**44**]. Nonetheless, an experiment has done by Tiwari, 2005 with implementing a low organic loading rate of 1.5 kg COD/m^3.d has indicated to successful granulation process with no disintegration in granules structure and size also [**45**]. Even though, organic loading rates of (2-4.5) kg COD/m^3.d has been recommended for high-quality growth of granules [**46**]. The variation of prior opinion has occurred as a result of process complication and the numerous factors that essentially affecting the granulation growth. It can believe that during start-up operation and granules formation, the implementation OLR values must be carefully located to obtain good growth.

7.3 pH and alkalinity

In general, neutral pH as well as elevated partial pressure of hydrogen is significantly essential for high-quality granules formation [**47**]. pH inside granules particle has been normally found to be inferior than in surrounding solution [**48**]. According to the nature of microorganisms, acidogenic microorganisms seems to be less sensitive in case of pH fluctuation than methanogenic microorganisms, whereby typical range of pH more than 6.3 is suitable for the latest kind. Practically, implementation of pH less than 6.3 will result in prevailing acidic formation which is mainly prevented the methanogens formation as well as methane production [**49**]. On the other hand, alkalinity plays a significant role on fluctuations neutralization in volatile fatty acids concentration which is always ready to arise due to the increase or variation in organic loading rate [**50**]. It is reported that the typical values of alkalinity have to be in the range of 250 to 950 mg/l [**51**].

7.4 Nutrient

The existing of nutrients such as nitrogen, phosphorus and sulfur in the influent is fundamentally needed to accomplish a successful development of granules. Basically, throughout the first phases of granules formation, surplus nutrients into influent can enhance the process and have no deadly effect. While lack of nutrients in substrate can adversely affect the granules formation process, whereby it has been reported that at a nitrogen concentration of less than 300 mg/l has resulted in granules growth dwindling [**51**]. In addition, over-elevated concentration of nutrients has resulted in process inhibition [**52**]. Nonetheless, nutrients advantages can be significantly demonstrated in preventing granules flotation as well as reducing the effect of shocks [**53**]. Considering that the composition of ammonia is totally synthesized as a result of nitrogen correlation with hydrogen to introduce electrons donor, it can be concluded that ammonia is playing a significant role on pH correction in medium [**54**]. Also it is important to mention that methanogens organisms are generally utilizing ammonia as a food source which is giving more acceleration for granules activities in the methanogenic phase.

7.5 Cations and heavy metals

Granules formation is a complicated process in which numerous of concepts have variously explained their generation. However, an adsorption and adhesion of bacterial is believed to form granules. An acceleration of granules formation have truly been observed in case of existence cations or addition through connection between diverse charge cells such involving their surfaces with ECP structure to produce bigger as well as stronger granules [**7**]. On the other hand, the major required fractions for metals on the bacterial surface are amino and carboxyl fractions in proteins [**55**]. The toxicities of some metals are completely independent on various factors such as category and structure of metal ion, pH, VFA concentration, HRT, and the strength of required fractions on the bacterial surfaces [**56**]. The literature has widely revealed the task of some specific multivalent cations on the granules formation such as calcium, aluminum and iron. Starting in calcium, whereby its role on the primary granules formation improvement is more than effective. Specifically, calcium enhances the adhesion process between the cells and the ECP, where it used as linking material [**17**]. Thus, the presence of calcium is necessary for the successful of granules growth. Various opinions related with the optimum concentration of calcium in solution have been proposed. A range from (80-150) mg/l was proposed to be the optimum concentration of calcium, on which granules formation in first phases is accelerated [**57**]. However, other opinion has indicated that the optimum concentration of calcium should be around (150-300) mg/l [**58**]. Another opinions by researchers have been broadly conducted to give various cases in the optimization of calcium. Although, the excess in calcium concentration may cause granules growth inhibition. In relation to the iron, strong evidences have indicated that a correlation of iron and COD conversion to biomass is in fact existed [**59**]. Supporting evidences has referred to the importance of excess of iron concentration up to 300 mg/l which is leading to obtain large granules size within shorter interval [**8**]. In addition, the aluminum has a drastic role on the acceleration of granules formation [**58**].

However, the overload of minerals existing in UASB reactor have absolutely led to process inhibition, and also caused ash formation instead of activated granules.

8. Bacterial activity to biogas

The production of methane in form of biogas includes the organic materials conversion under anaerobic conditions that is represented in wastewater treatment by employing UASB reactor. This process is mainly required some kinds of bacterial to be done. The conversion by bacterial can be achieved throughout the following phases: hydrolysis, acidogenesis, acetogenesis and methanogenesis. And depending on solution pH, and temperature [**60**]. Under low pH, no gas production can occur considering that at low pH the methanogens bacterial are not active, in addition of VFA accumulation phenomenon which is indicated to its gas production inhibition, the phases stability can be lost in this cases. On the other hand, temperature of reactor is playing a positive important role on the quantity of produced gas, whereby under psychrophilic range of temperature which can implemented with less than 20°C, the amount of biogas will be produced but limited. Under the thermophilic condition of (42-55) °C more biogas can be obtained. However in mesophilic temperature of (28-41) °C larger biogas generation can be done. It is believed that methanogens group of microorganisms are definitely responsible of organics biodegradation which is resulted in biogas production.

9. Conclusion

The key factor of the successful operation of UASB reactor is mainly granulation evolution. Series of accurate physico-biochemical interactions will lead to two objectives i.e. secondary wastewater treatment in high quality, and saving biogas in form of methane. Nowadays, granules can be used to treat various kinds of effluents with providing more safety as well as protection to our environment. It can be concluded that UASB reactor is the most attractive reactor on which high removal efficiency of organic materials can be achieved by preparing active microorganisms inside big granules can digest high strength organics wastewater. In relation to the ECP, it is found that ECP are very important factor on which microorganisms can be aggregated. ECP linkage with different charges have given granules strong bonding against washout. In the other hand, inorganic materials content have not shown interested role or contributing activities toward granules development. Also, it is worth mentioning that biogas process is mainly related to the developing granules activities. In terms of temperature and pH and their effective role on the biogas production, it can be recommended that adjustment temperature and pH is more than important to obtain successful as well as complete work in this field. Finally, the correct application of such a system like UASB reactor must be done carefully, taking into consideration factors that affecting the process.

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