

# Comparison of accelerated anaerobic granulation obtained with a bench-scale rotating bioreactor vs. a stationary container for three different substrates

ER Els\* and K Keet

Department of Process Engineering, University of Stellenbosch, Private Bag X1, Matieland, Stellenbosch 7602, South Africa

## Abstract

Anaerobic digestion is a very efficient technology for the treatment of wastewater from the food industries. The upflow sludge blanket reactor (UASB) is used to convert carbon in waste streams to CO<sub>2</sub> and CH<sub>4</sub>. The risk of a shortage of anaerobic granules in a situation that requires a replacement granule charge for the UASB is a barrier to implementation of anaerobic technology in countries without UASB reactors, producing a surplus of granules to provide for an adequate inventory of granules. Accelerated granulation provides a means to reduce this risk. Peach cannery effluent (PCE), sucrose and lactate were tested as possible substrates for accelerated granulation induction for un-granulated sewage sludge. Six experimental runs of 20 d each were done to explore granule growth. Each substrate was seeded with sewage sludge in a 5.4 l bioreactor rotating at 2 r/min, as well as in a stationary container. A rapid drop in pH occurred during the first few days of every run. A lower overall pH in the bioreactor indicates a higher bacterial activity relative to the stationary container. The big drop in pH initially with PCE and sucrose inhibited methanogenic activity and granule growth for these substrates.

The settleability of the final effluent from the reactor is superior to that of the stationary container. The total suspended solids analysis showed that the reactor produced a marked increase in the larger particulate sizes, indicating the positive contribution of the rolling action towards growth of granules.

**Keywords:** anaerobic digestion, granulation, wastewater, accelerated granulation

## Introduction

Anaerobic digestion is commonly used to treat wastewater from the food industry. These effluents have a high organic content and few toxic minerals are present. The nature of these effluents is such that it is often ideal for anaerobic digestion. The upflow anaerobic sludge blanket (UASB) reactor is used extensively for these applications as it is simple to operate, has a small footprint and a high hydraulic throughput. The UASB reactor converts waste carbon to gaseous products, of which CO<sub>2</sub> and CH<sub>4</sub> are the primary components (Gerardi, 2003).

The biogranules are the active ingredients in the reactor. A granule consists of different layers of micro-organisms. The digestion process by the different symbiotic layers in the granule can be explained by a staged organic digestion process (Britz et al., 1999):

- Exo-enzymes are secreted by the outer-layer micro-organisms
- These enzymes degrade complex carbohydrates into simple sugars which are absorbed and digested by the outer-layer organisms
- Digestion products from these species diffuse deeper into the granules where they are consumed by the acidogenic species
- The digestion products of these species then form the primary food source of the microbes populating the core of the granule, the methanogens

- The digestion products produced by these species exit the granule via the biogas vent.

A fraction of the carbon digested is converted into biomass, resulting in an increase in granule size. It can take up to 12 months to form granules of sufficient size to avoid washout from an upflow bioreactor (Agrawal et al., 1997).

Chen and Lun (1993) suggested that anaerobic sludge granulation starts with the formation of nuclei. The bacteria involved in the nucleus formation are *Methanosarcina* and *Methanothrix*. The methanogens must grow syntrophically with a wide spectrum of other bacteria, and these also play a very important part during the 2<sup>nd</sup> step, which is the process of a nucleus growing into a granule.

Fang (2000) investigated biogranules sampled from various UASB reactors. He found that the microbes were packed together. According to this researcher, the microbial distribution inside a UASB reactor is dependent on the degradation thermodynamics and kinetics of individual substrates. This researcher noticed that granulated degrading carbohydrates typically exhibit a layered distribution. The surface layer is populated with hydrolytic/fermentative acidogens, a mid-layer comprising syntrophic colonies and an interior layer comprising acetotrophic methanogens. Fang (2000) concluded from his observations that biogranules should be less vulnerable to the changes of the mixed liquor condition, because the large majority of microbes inside the biogranules are shielded from the mixed liquor environment. Methanogens are very fragile organisms that occur in the centre of the granules.

Hydrogen is an inhibitor to the granulation process. The pH level plays a vital role in the biogranulation process, as at a pH of lower than 6.5 a decrease in activity starts to occur in the methanogenic species, which plays a vital role in granula-

\* To whom all correspondence should be addressed.

☎ +27 21 808 4486; fax: +27 21 808 2059;

e-mail: [rels@sun.ac.za](mailto:rels@sun.ac.za)

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tion. Reduced methanogen activity causes a further increase in hydrogen concentration and eventually causes the digester to sour, see Nazaroff and Alvarez-Cohen (1991). Van Zyl (2004) also reported that lower pH decreases gas production from the reactor. Should the pH drop to below 6.5, it should be controlled by adding a suitable form of carbonate until the pH is above 6.5. Van Zyl (2004) suggested the use of  $\text{CaCO}_3$  as the  $\text{CO}_3^{2-}$  acts as a buffer and the  $\text{Ca}^{2+}$  enhances flocculation due to increased production of extra-cellular polymers, which in turn act as a hydrogen sink that stabilises the pH.

The upflow arrangement in the UASB requires the use of granules of sufficient size to prevent washout from the reactor. Anaerobic granule growth is very slow; it takes months for granules to grow in diameter to usable size, say bigger than 0.5 mm. This time lag is a barrier in reactors operating in Third World countries with the risk of a shortage of anaerobic granules in a situation that requires a replacement granule charge for the UASB reactor in the event of, e.g. poisoning.

### Accelerated granulation

Research done by Britz et al. (2002) on accelerated granulation, seeded lactose, glucose and sucrose substrates with anaerobic sludge and incubated it in shaking water baths over a period of 14 d. An increase in granulation, from sludge to individually visible granules, was observed for the glucose and lactate, but not for the sucrose. It was concluded that the increase in granule formation indicated that granulation may be enhanced in batch systems over a shorter period and that the granulation process is characterised by a rapid drop in pH at the start (daily addition of base (KOH) for pH control is necessary), resulting from an increase in propionic and acetic acids. This is followed by a subsequent increase and stabilization of pH as the methanogens become active and start to consume the hydrogen. Methane is released by methanogens in the sludge or granules. If methane is produced it is proof that the granules are indeed active in the reactor. It was concluded from the study that anaerobic biogranules can be cultured in lab-scale batch reactors, starting with secondary, stabilised non-granular anaerobic sewage sludge, on condition that the proper environmental parameters to create bacterial stress are in place. Bacterial stress conditions are created by providing a daily shock in the form of fresh carbon substrate, combined with the dynamics of flasks in a shaker tray. These conditions provided for accelerated granule growth to obtain granules of useable size in a period of 14 d.

Commercialisation turned out to be problematic with the above-mentioned method as the shaking flasks did not facilitate scale-up to industrial size reactors (Van Zyl, 2004). The Water Research Commission (WRC) of South Africa funded a research project with the aim to develop a reactor of sufficient volume to replace the granules of an industrial-size UASB sludge bed, within 20 d (Van Zyl, 2004). Two 4.5 l bench-scale bioreactors were designed to try and simulate the motion induced by the shaker baths causing increased granule growth rate. These bioreactors had the added advantage of facilitating scale-up and the study thereof. This project proved that granulation can be achieved in a small reactor vessel from dispersed anaerobic digester sludge. However, it was obvious that the system was far from optimised (Els et al., 2005).

A 3<sup>rd</sup> test reactor was designed to imitate roller table movement in an attempt to increase the 20 d useable granule yield from the reactor. The 5 l reactor consists of a rotating shell with baffles. The optimal rotational speed for the reactor was determined at 2 r/min. This reactor improved the granule yield. Based

on the findings of this study it was recommended that due to the many variables present, a further optimisation study should be done on a granulation reactor of one order of magnitude larger. According to Van Zyl (2004) it would provide more confidence and information before a scale-up to a commercial size reactor was attempted.

The experimental work done to date on accelerated granulation used only PCE as a substrate. Various carbon sources can, however, be used as carbon source for granulation. This study explored the effectiveness of three different substrates for accelerated granulation.

### Experimental

The presence of high-energy value sugars is paramount during the growth of granular sludge. The substrate, or food, used for test work therefore should contain sufficient amounts of sugars (in the order of 2 000 mg/l) to ensure granule growth.

Three substrates were tested for biomass retention, granulation enhancement and methanogenic activity in a rotating bioreactor as well as in a stationary container. The substrates used were peach cannery effluent (PCE), sucrose and lactate. After preparation of the substrates, each one was diluted with distilled water to 2 000 mg/l COD for experimental purposes. Each test was run for a 20 d period. The substrate was replaced every 24 h. The experimental runs for each substrate were performed in parallel in the bioreactor and the stationary container was concurrently run in parallel.

#### Substrate preparation

##### *Peach cannery effluent (PCE)*

Peach cannery effluent (PCE) was obtained from a local cannery, and used as substrate for the 1<sup>st</sup> run with both the bioreactor and the stationary container. The chemical oxygen demand (COD) of the PCE was 17 800 mg COD/l. The peach effluent is filtered through 250 µm filter disks as part of the preparation, to remove the bulk of the solids. However, a great amount of solids remained in the substrate, and the substrate had a cloudy orange colour as a result. The PCE is available locally from industries free of charge, which would make it an ideal alternative to costly substrates.

##### *Sucrose*

The sucrose substrate is a synthetic substrate prepared by a blend of the ingredients listed in Table 1.

Component	Concentration g/l
Peptone	0.4
Sucrose	1.4
Meat extract	0.3
Lactate	0.2
$\text{NH}_4\text{Cl}$	0.3
$\text{KH}_2\text{PO}_4$	0.1
$\text{NaHCO}_3$	1.2
$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	0.02

The procedure for preparation of the substrate was taken from Show et al. (2004). The peptone, sucrose, meat extract, lactate,  $\text{NH}_4\text{Cl}$  and  $\text{KH}_2\text{PO}_4$  were mixed with 4.8 l of distilled water. The  $\text{NaHCO}_3$  was dissolved separately in 10 ml of distilled water and then added to the 4.8 l mixture. The mixture, as

well as a separate bottle with 200 ml distilled water, were autoclaved for 2 h and then cooled. Before use, the  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  was first dissolved in the 200 ml of distilled water and then added to the 4.8 l mixture. The substrate was then ready for use.

### Lactate

Lactate substrate was obtained from a yeast extract. The substrate was prepared by mixing the ingredients listed in Table 2.

Component	Concentration g/l
Yeast extract	5
Peptone	2
$\text{KH}_2\text{PO}_4$	10
Sodium lactate	10
Tween 80	1.2
KOH	pH 7.00

The ingredients were added to 5 l of distilled water. A magnetic stirrer was used to dissolve the ingredients; the pH was then adjusted to 7 by adding approximately 160 ml of KOH (with a concentration of 2 molar). The prepared substrate was then autoclaved for 2 h and then cooled to room temperature before it was used in the reactor or the stationary container, as the bio-population should not be exposed to the high temperature.

### Sludge preparation

The micro-organisms used for the experimental work were obtained from a local municipality in South Africa, Kraaifontein Sewage Works. The un-granulated sludge had already been treated aerobically. Particles above 2 mm in size were sieved from the sludge.

### Preparation of the bioreactor and the stationary container for an experimental run

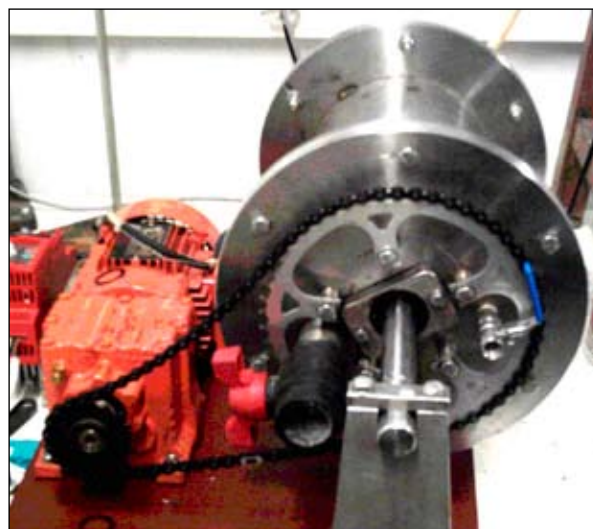
The bioreactor and container were kept in an isothermal room at 35°C. The bioreactor, see Fig. 1, consisted of a rotating shell with baffles. Gas was released via a gas vent through the stationary shaft of the reactor (Van Zyl, 2004). Before each run, the reactor was cleaned with water only (soap should not be used to avoid the risk of poisoning the bacteria). Thereafter, the rubber seals were replaced on each side of the shaft. The side flanges of the reactor vessel were then secured and fastened with bolts. The complete reactor set-up and internal baffles are shown in Figs. 1 and 2.

Before each run, the reactor should be filled to its capacity with distilled water and run for 24 h to test for leaks. The gas outlet port from the reactor is connected to an off-gas flow-measuring unit.

The 'stationary container' was merely a plastic container. The feed materials were kept the same as for the reactor. The lid was closed, but provided for biogas to be released with an increase in pressure.

### Substrate change (every 24 h)

Based on previous work done (Els, 2005) on accelerated granulation, the sludge was mixed with substrate in a 1:3.5 ratio on the 1<sup>st</sup> day of a run. The bioreactor was filled to 5.4 l substrate capacity. Therefore, the mixture comprised of 1.2 l of sludge



**Figure 1**  
*Bioreactor with motor drive*



**Figure 2**  
*Bioreactor shell*

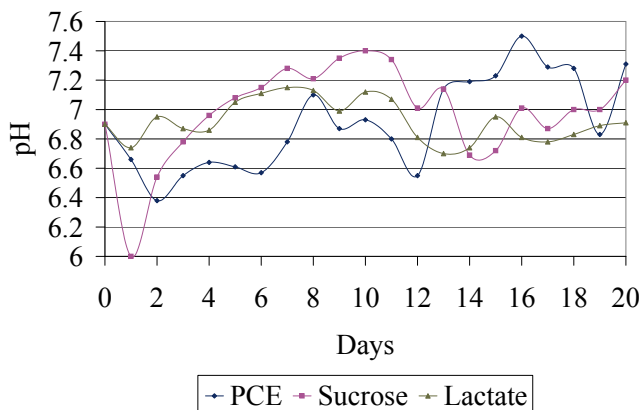
and 4.2 l of substrate.

Each day after start-up (Day 1 up to Day 19), the bioreactor was stopped for a period of approximately 40 min. The sludge mixture inside the reactor was allowed to settle for 20 min, in order to minimise the amount of solids lost through wash-out on drainage. A volume of 1.2 l of used substrate, an almost clear mixture, was then tapped from the bioreactor side outlet valve. The pH was measured. When the pH was below 6.5, extra bicarbonate was added to the reactor with the fresh 1.2 l substrate. When the pH was above 6.5, only the fresh substrate was fed to the reactor.

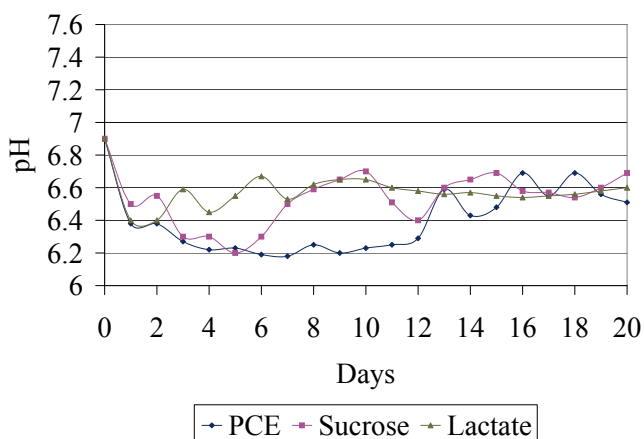
The same principle was followed with the stationary container. The 1.2 l of used substrate was poured out of the container; the pH was measured and then replaced with fresh substrate. The content of the stationary container was stirred briefly by hand after substrate replacement.

### Results and discussion

Six experimental runs were performed of which two runs were done in parallel with the same substrate in the bioreactor and also in the stationary container.



**Figure 3**  
Substrate pH for the stationary container



**Figure 4**  
Substrate pH for the reactor



**Figure 5**  
Settleability for PCE

### pH results

During each experimental run the pH of the substrate was measured daily. The log for the pH values obtained with each of the 3 substrates tested in the container can be seen in Fig. 3, and Fig. 4 shows the same information for the reactor.

The pH levels of all the runs varied from day to day. However, relative to the rest, the daily pH of the lactate (the 3<sup>rd</sup> run) seemed to be the most stable for the container. The sucrose and

the glucose (PCE) substrates caused a larger initial drop in the pH than the lactate substrate. This tendency was due to the production of acetic and propionic acid, which caused the pH to decrease steadily. The low pH then recovered and increased to above pH 6.5 with the methanogens consuming the acetic acid to form methane and carbon dioxide.

For the lactate substrate there was a smaller initial drop in pH and more pH stability. The smaller drop in pH was due to the lactate, an acid providing feedstock to the methanogens which become active more quickly, consuming hydrogen. Hydrogen is also consumed for the production of extra-cellular polymers (ECP).

On the same scale Fig. 4 illustrates curves for the reactor pH. The overall pH is lower than that of the container. The lower pH is due to mixing in the reactor enhancing the bacterium-to-bacterium, as well as the bacterium-to-nuclei contact (Van Zyl, 2004).

During the 1<sup>st</sup> two runs, with PCE and sucrose, the pH was not adequately controlled for the first few days, at which stage the methanogenic population had been inhibited due to a too low pH. During the course of the 2<sup>nd</sup> run, a leak from the reactor was identified and the reactor had to be drained to repair a rubber seal. For this period the reactor content was exposed to air. The stationary container content was also exposed to air as the lid was removed every day when the substrate was replaced.

The pH of the 3<sup>rd</sup> run was most stable and remained above 6.5 after 5 d. From the results obtained it could be concluded that without methanogenic activity the pH should be monitored and controlled effectively, especially during the first 5 d; once the methanogens were active the pH remained stable between 6.5 and 6.7. This observation was also confirmed by Van Zyl (2004).

### Settleability

Settleability of granules is a requirement for the UASB reactor to prevent the loss of granules. If the anaerobic granules do not settle fast enough once wastewater is pumped from the bottom through the granules in the UASB reactor, the active granulated solids will be carried upward with the wastewater and washed out in the reactor overflow. The purpose of the bioreactor is to enhance granulation by producing active, settleable solids that can be used in a UASB reactor instead of normal untreated sludge. The same volume of settled solids, taken from the Day 20 effluent, was shaken up in a small container. The samples were given 30 min to settle before photos were taken. The photo in Fig. 5 shows the settled effluent from the PCE run. The bottle to the left shows the effluent from the stationary container; the right-hand side bottle shows the reactor effluent.

No settling, as indicated by a clear solution on top, was obtained for the stationary container after 30 min. The bottle on the right illustrates that the solids in the effluent from the reactor were much more settleable.

The photo in Fig. 6 shows the settling obtained during the sucrose run; the container on the left was for the stationary container. The leak during Run 2, the sucrose run, limited the amount of solids produced in the reactor. The solids in the reactor container (to the right) contained a 3<sup>rd</sup> volume of solids; however, the better clarity of the liquor indicated superior settleability.

The concentration of solids in the lactate run was too high to notice any separation of solids in 30 min (see Fig. 7).

From Day 14 biogas in the stationary container attached itself to the solids, forcing the solids to form a layer on the top; this is shown in Fig. 8. The layer of solids was visible up to Day



**Figure 6**  
Settleability for sucrose



**Figure 7**  
Settleability of lactate

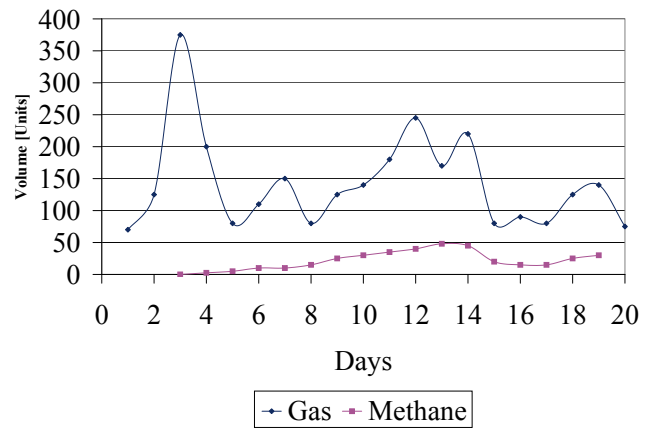


**Figure 8**  
Solids in lactate

20 and caused a large amount of solids to be washed out during substrate replacement.

### Gas production

The off-gas from the reactor was monitored by means of a purpose-built gas flow meter. The meter consisted of a water-filled U-tube. The reactor gas outlet was connected to the U-tube via a 3-way valve. The rise of a float in the U-tube switched a 3-way valve to vent the biogas. An electronic counter added up the switch cycle counts of the valve. Samples were taken from



**Figure 9**  
Biogas and methane production during lactate run

the connecting tube with a syringe and analysed with a gas chromatograph for the methane content.

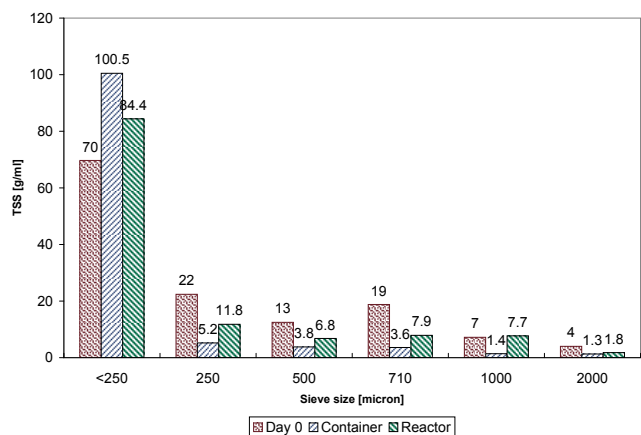
Although gas was produced during the PCE and sucrose runs the gas did not contain any methane due to the low pH. For the 3<sup>rd</sup> run with lactate the gas production can be seen in Fig. 9.

The figure qualitatively illustrates that the methane production follows on the gas production. During the first 4 d no methane was produced

### Total suspended solids

The size distribution of the effluent was done on the day a run ended. Five size fractions were separated using a series of sieve trays sized: 2 mm, 1 mm, 710  $\mu\text{m}$ , 500  $\mu\text{m}$  and 250  $\mu\text{m}$ . The size distributions were done in triplicate to improve accuracy; 25 ml aliquots from the sludge effluent of the reactor and the stationary container after each run were used. The effluent was well mixed before a sample was taken.

The total suspended solids (TSS) were obtained as the weight gain on the filters after the samples had been filtered and dried in an oven at 105°C for 5 h. Due to the pH drop in the PCE and sucrose runs, with resulting inhibition of methanogenic activity, only results for Run 3 with lactate as substrate, are shown in Fig. 10. The TSS values of Day 0 sludge can be seen for the different size fractions along the horizontal axis. The TSS values of the solids on completion of Run 3 with the stationary container



**Figure 10**  
Total suspended solids size fractions for the lactate run

and reactor are shown alongside, to the right-hand side, of Day 0 TSS for the different size fractions. It is evident that from 250 µm upward the TSS values of the reactor exceeded those of the stationary container for each size fraction, except for the 2 000 µm fraction, which was very small. It is evident that the bioreactor was more effective in producing larger granules due to the rolling and compacting motion in the reactor.

Insert Figure 10.

## Conclusions

A drop in pH from 7 to below 6.5 in one day occurs as the microorganism community is initially supplied with an easily degradable carbon source. There is a time lag in the food chain for the consumption of the hydrogen produced by the methanogens resulting in a sharp drop in pH. During this period it is vital to increase the pH by addition of base/alkali to prevent the inhibition of the methanogens. Overall the reactor remained at a lower pH than the stationary container, indicating a higher bacterial activity. The initial drop in pH for the reactor is higher and the reactor pH did not recover with small additions of CaCO<sub>3</sub>, indicating a high rate of acid production. The lactate substrate shortened the metabolic path for the methanogens, resulting in better pH control. Once the methanogens are active the pH becomes self-regulating. The low pH for the PCE and sucrose runs caused inhibition of the methanogens with resulting limited granulation; as a consequence no preference between the effectiveness of the reactor vessel vs. the stationary container could be established with regard to accelerated granulation for these two substrates.

The settleability of the reactor effluent is superior to that of the stationary container. This should be attributed to compaction of the granulate as a result of the falling and rolling action within the reactor. The final TSS of the reactor showed a marked increase in the larger size fractions over those of the stationary container. Although not specifically proven here, ECP production in combination with the dynamics of the rolling and falling action should assist in the formation of larger granules in the reactor.

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