Comparative Study of the Power Consumption on the Production of Xanthan Using the Traditional Industrial Stirred Tank Reactor and a Novel Oscillatory Baffled Reactor

Ebtihaj Jambi, Xiong-Wei Ni, Brian McNeil, Amal Basaleh and Linda Harvey

King Abdulaziz University, Girls section, Faculty of Science, Biochemistry Department
ejjambi@kau.edu.sa

Abstract: The production of xanthan gum by the bacterium *Xanthomonas campestris* (*X. campestris*) in a defined medium was studied using two different mixing pattern reactors the stirred tank reactor (STR) and a novel oscillatory baffled reactor (OBR). The purpose of this study was to calculate the power consumption ofthe operating reactors, different aeration and mixing speed conditions with different parameters were applied in the two reactors. The results of the power consumption of all conditions applied in the study were compared with the production of xanthan gum of each condition. The production of xanthan gum in the OBR showed relatively similar results to those of the STR with lower power consumption.
[Ebtihaj Jambi, Xiong-Wei Ni, Brian McNeil and Linda Harvey. **Comparative Study of the Power Consumption on the Production of Xanthan Using the Traditional Industrial Stirred Tank Reactor and a Novel Oscillatory Baffled Reactor.** *Life Sci J* 2013; 10(4): 2241-2249]. (ISSN: 1097-8135). http://www.lifesciencesite.com. 299

Keyword: Oscillatory baffled reactor (OBR), stirred tank reactor (STR), xanthan gum, power consumption

1. Introduction:

Xanthan is a well-known extracellular polysaccharide, produced and synthesised intracellularly by a gram negative bacterium *Xanthomonas campestris* (*X. campestris*) in an aerobic condition (Jarman and Pace 1984; Blanch *et al.,* 2008). Solutions of xanthan exhibit high viscosities and non-newtonian behaviour even in a small concentration (Yoshida and Tanner 1993). Native xanthan is a hetero-polysaccharide consisting of a cellulosic backbone with a penta-saccharide repeating unit. The main chain consists of two β-D-glucose units linked at the 1-4 position but the unique character of xanthan gum is derived from the tri-saccharide side chain. This chain is composed of (β-D-mannose-(1,4)-β-D-glucuronic acid-(1,2)-α-D-mannose) linked at the C-3 position to every other glucose residue in the main chain. Approximately half of the terminal D-mannose unit contain a pyruvic acid residue linked via a keto group to the 4 and 6 positions, with an unknown distribution; the internal mannose unit linked to the main chain may contain an acetyl group at the position C-6. The repeating unit of xanthan gum is shown in Figure 1.1 (Becker *et al*., 1998; Garcia-Ochoa *et al*., 2000 and Steinbuchel and Rhee 2005).

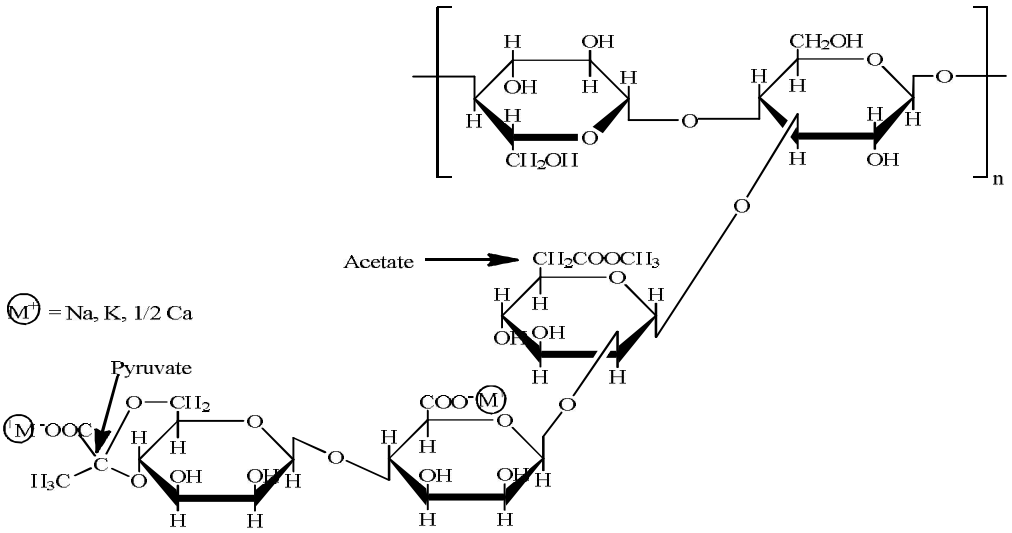


Figure 1.1: The repeating unit of Xanthan Gum.

The superior properties of xanthan gum have enabled it to compete with most of natural gums and also become the preferred product for many applications for a number of important reasons including its ability to impart a high viscosity solution, and high pseudoplasticity (Yoshida and Tanner, 1993). The high viscosity of solutions, water solubility of the polymer, emulsion stabilization, temperature stability up to 90 °C and pH 2–11, the compatibility with food ingredientsand its property of the addition of a small concentration of xanthan gum used in food products enables it to confer the required properties without affecting the taste of the final product have created important applications for xanthan in industrial food and non-food sectors as well as in oilrecovery (Lee, 1996; Sanderson 1981; Garcia-Ochoa *et al*., 2000; Rosalam and England, 2006). Table 1.1 lists some current uses of xanthan gum. In the agricultural industry, the unique rheological properties of xanthan has been used to improve the suspension of the solid component increasing flow-ability, reducing drift and increasing cling and permanence in herbicides, and insecticides.

Table 1.1: Main industrial applications of xanthan gum (Garcia-Ochoa *et al*., 2000).

Application	Functionality
Food industry	
Salad dressing	Emulsion stabilizer; suspending agent, dispersant
Dry mixes	Eases dispersion in hot or cold water
Syrups, topping, relishes, sauces	Thickener; heat stability and uniform viscosity
Beverages (Non-fat dry milk)	Stabilizer
Dairy products	Stabilizer, viscosity control of mix
Baked goods	Stabilizer, facilitates pumping
Frozen foods	Improve freeze-thaw stability
Pharmaceuticals	
Cream and suspensions	Emulsion stabiliser; uniformity in dosage formulation
Cosmetics	
Denture cleaners, shampoo, lotions	Thickener and stabilizer
Agriculture	
Additive in animal feed, pesticide formulations	Suspension stabilizer; improved spry ability, reduced drift, increased cling and permanence
Textile printing and dyeing	Control of rheological properties of paste, preventing dye migration
Ceramic glazes	Prevents agglomeration during grinding
Slurry explosives	Thickens formulation, improve heat stability (in combination with guar gum)
Petroleum production	Lubricant or friction reduction in drill-hole
Enhanced oil recovery	Reduces water mobility by increasing viscosity and decreasing permeability

In order to meet the challenges of producing environmental friendly products, xanthan gum has been introduced in the formulation of new generations of thermo-setting coatings due to its ability to disperse and hydrate rapidly (Rosalam and England, 2006). In the oil recovery approximately two barrels of oil remain in the ground for every one barrel produced (Rosalam and England, 2006). Because xanthan gum has excellent compatibility with salts, and resistance to thermal degradation, xanthan is useful as an additive in drilling fluids in the petroleum industry. In addition xanthan gum is used in oil drilling, fracturing, pipeline cleaning and in enhanced oil recovery EOR. In EOR xanthan gum is used in tertiary oil recovery operations. The function of xanthan is to reduce the mobility of injected water by increasing its viscosity (Nasr *et al.,* 2007). The basic principle is to apply polymer-thickened brine to drive the slug of the surfactant through a porous reservoir rock to mobilise residual oil and improve the separation of water and oil, thereby increasing oil recovery (Byong, 1996; Marudova-Zsivanovits *et al.,* 2006).The other applications of xanthan are removing rust, welding rods, wet slag, and cleaning other debris from gas pipelines. Many more application of xanthan gum can expected to be developed.

Traditionally the production of xanthan has predominantly been performed in stirred tank reactor STR. To improve mixing and hence mass transfer rate, especially in viscous non-Newtonian fluids, a large number of reactor types have been reported corresponding to the large number of industrial

fermentation process. Most commonly used bioreactors are bubble columns and air lift reactors.

Agitation as well as aeration play an important roles in the fermentation of xanthan due to the nature and the elaboration of exopolysaccharide in the culture of Xanthomonas campestris, the broth become very viscous and non-Newtonian and this leads to poor mixing conditions and limits mass, momentum, oxygen and heat transfer in the STR (Moo-Young 1985).

The oscillatory baffled reactor OBR a relatively new reactor is based on a methodology of superimposing fluid oscillation into a cylindrical tube containing periodically spaced orifice baffles, offering certain advantages in enhanced and uniform mixing with a very low shear rate compared with conventional mixing vessels. The oscillation interacts with each baffle to form vortices, providing significant radial motion in the column promoting better mass transfer.(Gaidhani *et al.,*2005).

In financial terms, the capital costs for manufacturing technologies are marginal to the overall operational profits. In this study we aimed to compare the performance of two different fermenter types the stirred tank reactor STR and a novel oscillatory baffled reactor OBR when used for a high viscosity process such as xanthan. In addition, to quantitatively analyse and describe the power consumption and the yield in certain conditions in both OBR and STR fermenters.

2. Materials and Methods

2.1 Microorganism

The microorganism used in this study was wild type *Xanthomonas campestris*ATCC 13951(*X. campestris*) supplied as a lyophilized culture. The culture was resuscitated by addition of sterile diluent and then plated out onto yeast malt (Oxoid Ltd, Basingstoke) plates, andincubated for 24 hours at 30°C.Inoculum were produced by transferring asingle growing colony of*X. campestris* from 24 hours old *X. campestris* plates to 200 ml of sterile YM broth in a 500ml Erlenmeyer flask and incubated at 200rpm and 30°C for 24 hours, from which standardised inoculums was derived. An optical density of 1.60 (λ 600nm) was found to be equivalent to approximately 2.2 x 107 cells. A 10% v/v inoculum was used to seed the fermenter for the production of xanthan gum. The composition of the standard medium was in (g per litre) is: citric acid 2.1, NH_4NO_3 1.144, KH_2PO_4 2.866, $MgCl_2.6H_2O$ 0.507, Na_2SO_4 0.089, H_3BO_3 0.006, ZnO 0.006, $FeCl_3.6H_2O$ 0.0024, $CaCO_3$ 0.020 and Extra-pure 37% HCl 0.13ml. The pH was adjusted to pH 7.0 with 1M NaOH. 20g/l of glucose was autoclaved separately. For all experiments, temperature was 30°C.

2.2 Analytical methods

Biomass was measured as dry cell weight. The fermentation broth was diluted up to 5 times and centrifuged at 10,000rpm for 30 min. The supernatant was used for the estimation of glucose and xanthan gum. The cell pellet was re-suspended and washed in 20 ml distilled water at 10,000rpm for 15 min twice. The pellet was then transferred to a pre-weighted Eppendroff tube and centrifuged for 5 min at 10.000 rpm, after which the cell pellet was dried in an oven at 80°C for 24 hour then was weighed after cooling in desiccator for 2 hour. Polysaccharide estimation was obtained by dry weight measurements precipitated by adding 5 volumes of iso-propanol to cell free fermentation broth. The precipitate was then filtered through a pre-dried pre-weighed glass microfibers filter paper disk range MF200 (Fisher Scientific). The precipitated biopolymer and filter disc was then dried in a microwave for 25 min at thaw setting. Followed by oven drying at 80°C for 30 min, then cooled in the desiccator and weighed. Glucose was determined using an enzymatic/ UV determination assay kit (R-Biopharm)

2.3 Reactors

2.3.1 Stirred Tank Reactor (STR)

The main fermenter used was a glass BioFlo-3000 (New Brunswick Scientific (UK) Ltd, St Albans, Hertfordshire, UK)Figure 2.1.

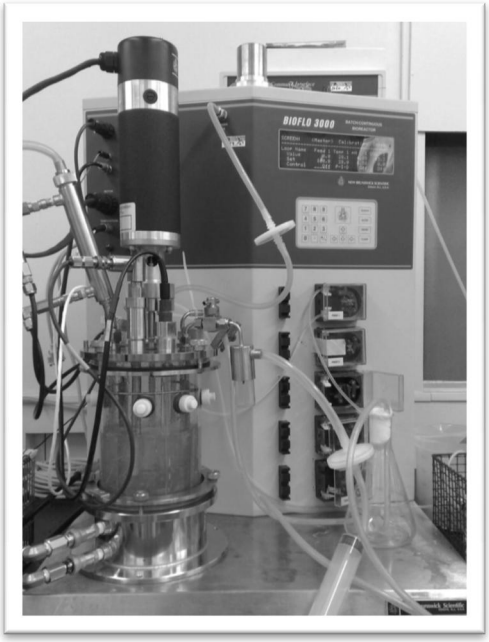
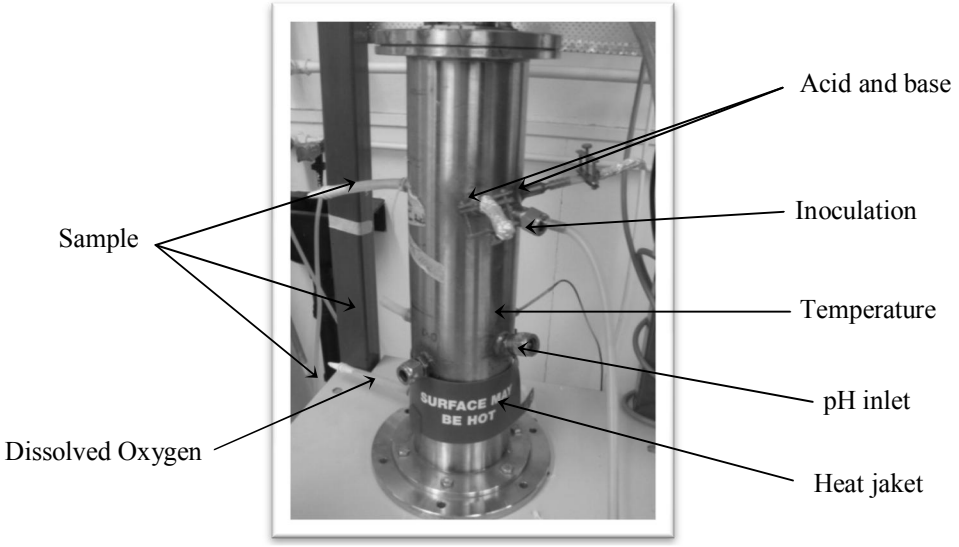


Figure 2.1: Operational configuration of the New Bruswick BioFlo 3000 STR during batch fermentation of X. campestris ATCC 13951.

2.3.2Oscillating baffled reactor (OBR)

A schematic diagram of the OBR setup is shown in Figure 2.2. It consists of a stainless steel column of a total liquid capacity of 3.3l. The working fluid capacity is 2l. Two side ports were provided for housing a dissolved oxygen probe and a pH probe. In addition, there is a port for a temperature probe as shown in Figure 2.2. A set of three orifice stainless steel plate baffles designed to fit closely to the wall of the reactor was used in this study. The baffles were equally spaced apart and supported by two stainless steel rods Figure 2.3. The baffle set is connected to the shaft of a piston through a supporting plate and driven by an electrical motor together with an inverter. The oscillation frequency, ranging from 0.2 to 10Hz, can be controlled using a speed controller. The amplitude of oscillation of between 5 and 30mm can be generated by the eccentric distance between the fly arm and the crank of the motor Figure 2.4.For sampling, three sample lines were fitted to the column at different heights. The parameters and conditions in both STR and OBR are shown in Table 2.1.



Figur ports.

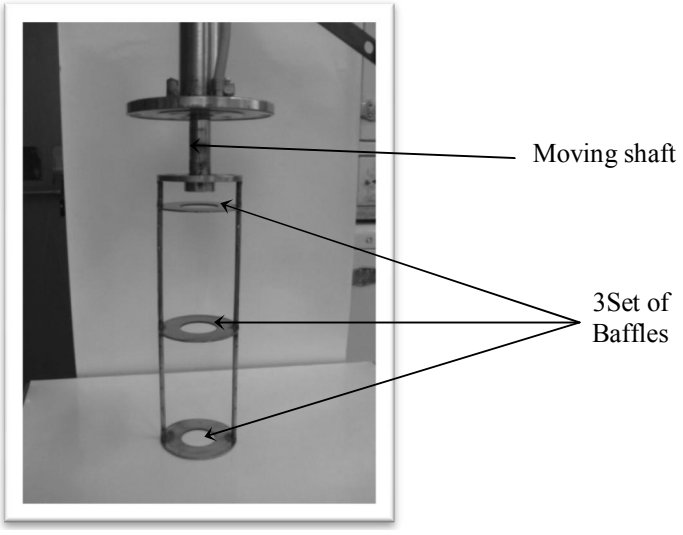


Figure2.3: A set of three orifice baffles and the supporter

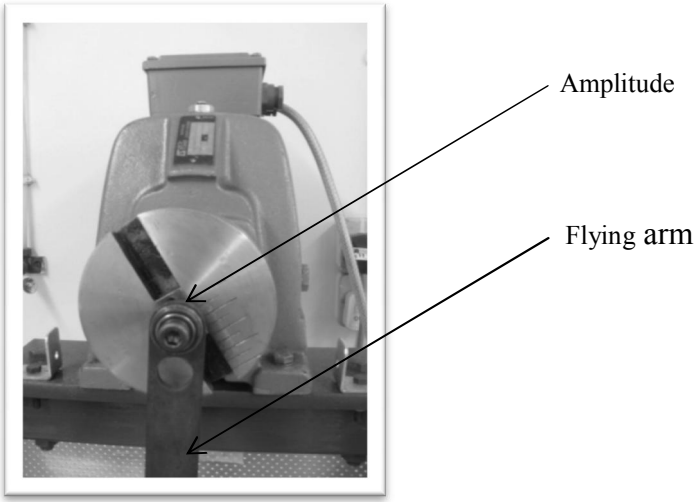


Figure 2.4: The motor and the adjustable amplitude

Table2.1: Operating parameters for both the STR and the OBR reactors

	2-liter STR fermenter	**2-liter OBR fermenter**
Total volume (l)	3.3 l	3.3 l
Operating volume (l)	2.0 l	2.0 l
Working volume (l)	1.3 l	1.3 l
Total height (mm)	248 mm	420 mm
Liquid height (mm)	98 mm	165 mm
Diameter of the vessel (mm)	130 mm	100 mm
Number of baffles	4	3
Width/diameter of baffle (mm)	Width 15 mm	Orifice diameter 46 mm
Sparger type	Ring sparger	Ring sparger (upward)
Aeration rate (vvm)	1vvm and 2vvm	1vvm and 2vvm
Agitation type	Two six-bladed Rushton turbines	Three stainless steel annular baffles
Agitation speed	500 rpm and 200 rpm	25 mm 2 Hz and 5 mm 5 Hz
pH	Uncontrolled	Uncontrolled
Temperature (°C)	30 °C	30 °C

2.3.3 Power consumption

For the estimation of the power density in the STR an equation of the form below was used (Rushton *et al.,* 1950).

$$P/V = \frac{P_0 \rho N^3 D_s^5}{V_v} \left(W/m^3\right) \quad (1)$$

where

P_0 = Number power of the impeller (Gimbun *et al.*, 1993) = 4.5

ρ = Density of the fluid (Kg/m^3)

N = Rotational speed of the impeller (rpm)

D_s = Diameter of impeller (m)

V_v = Volume (m^3)

For the estimation of the power density in the OBR a quasi-steady flow model equation form was used (Ni and Mackley, 1993).

$$P/V = \frac{2\rho N_b}{3\pi C_D^2} \frac{1-\alpha^2}{\alpha^2} x_o^3 (2\pi f)^3 \left(W/m^3\right) \quad (2)$$

where

f = Frequency (Hz)

x_o = Amplitude (m)

α = Ratio of effective baffle area to tube area (Ni and Goa, 1995) = 0.212

ρ = Density of the fluid (Kg/m^3)

C_D = Discharge coefficient (Ni and Goa 1995) = 0.7

N_b = Number of baffles per unit length

3. Results and Discussion

3.1 Effect of the combination of aeration and frequency and amplitude in the OBR and agitationin the STR on the polysaccharide production.

In general, the fermentations carried out at high gassing rate (2vvmat 5 and 25mm amplitude) considerably outperformed those carried out at lower gassing rate (1vvm at 5 and 25mm). Therefore, in terms of xanthan production although amplitude clearly had an influence, this was complicated by the dominant effect of gassing rate upon xanthan synthesis. The results for the effect of the combination of aeration and frequency and amplitude upon xanthan production under these operating conditions in the OBR are shown in (Figure 3.1).It can be seen that broadly speaking the pattern of xanthan production was not influenced by aeration rate only within the first 72 hours of the fermentation but it was influenced by a combination of higher amplitude and high aeration. After this point, at high aeration rate, xanthan synthesis continued to the process end with a significantly higher xanthan yield at higher aeration rate process.

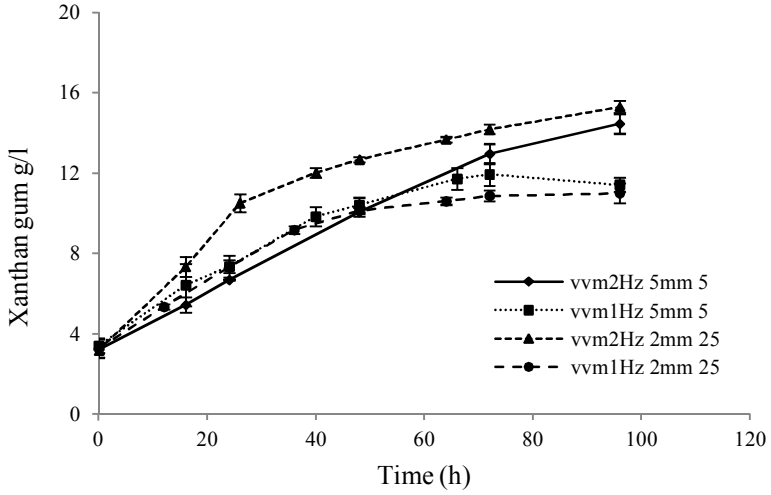


Figure 3.1: Effect of the interaction between aeration, frequency and amplitude on xanthan gum production in batch cultures of X. campestris ATCC 13951 in OBR at 30 °C.

On the other hand, the effect of the increasing of the aeration in the STR was significantly showed at the agitation speed 200rpm Figure 3.2. All processes started with some xanthan gum present that came from the inoculum. Production of xanthan gum initially followed a very similar trend in all these processes with the exception of the 200rpm 1vvm process.

The maximal concentration of xanthan gum from the STR in this study was 15.8 g/l at 96 hours in the process operated at 500rpm and 2vvm. Formation of xanthan gum was found to be similar in two different conditions of aeration and agitation, that is, 200rpm and 2vvm (low agitation with high aeration) and 500rpm and 2vvm (high agitation with low aeration), where the maximum xanthan in these two processes was 14.25g/ l and 15.08g/l respectively. These results suggested that at high aeration rate the influence of the agitation speed is decreasing. At 200rpm and 1vvm (low agitation with low aeration) the maximum production was only 9.56 g/l by the end of fermentation at 96 hours.

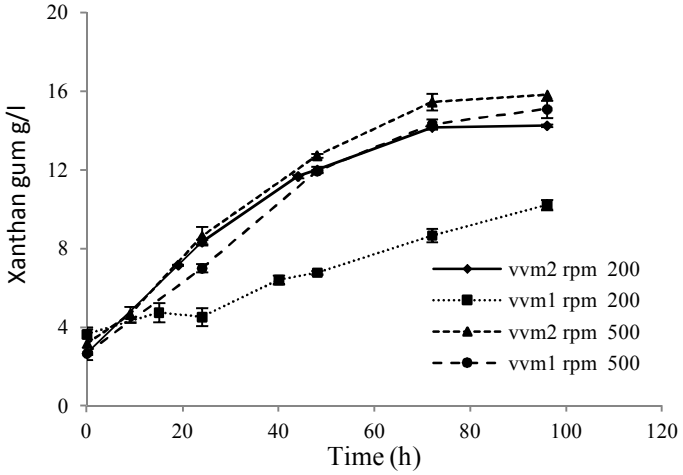


Figure3.2: The Effect of the interaction between aeration and agitation speed on xanthan gum production in batch cultures of X. campestris ATCC 13951 in the STR at 30 °C.

3.2 Effect of the combination of aeration and frequency and amplitude in the OBR and agitation in the STR on the power consumption

Figure 3.3 shows the power density under different operational conditions used in this study of OBR vs. the yield after 96 hours. A clear effect of aeration rate is apparent in this figure at both high and low amplitudes. Yield rises significantlyfrom 11.9 g/l to 14.45 g/l at low amplitude 5mm with power density of 0.44kW/m^{3}with increasing the aeration rate and it was close to the production of the polysaccharide at high frequency 25mm at the same aeration rate with power density of 3.532kW/m^3. By contrast at low gas rate, thus effect is less apparent. At 500rpm, the highest power density of 2.321kW/m^3 was achieved. The aeration showed no effect on the power density in this equation, whereas at lower agitation rate 200rpm the aeration does not affect the power density but clearly affect the production of xanthan gum from 9.56 g/l to 14.25 g/l with power density of 0.148kW/m^3 Figure 3.4.

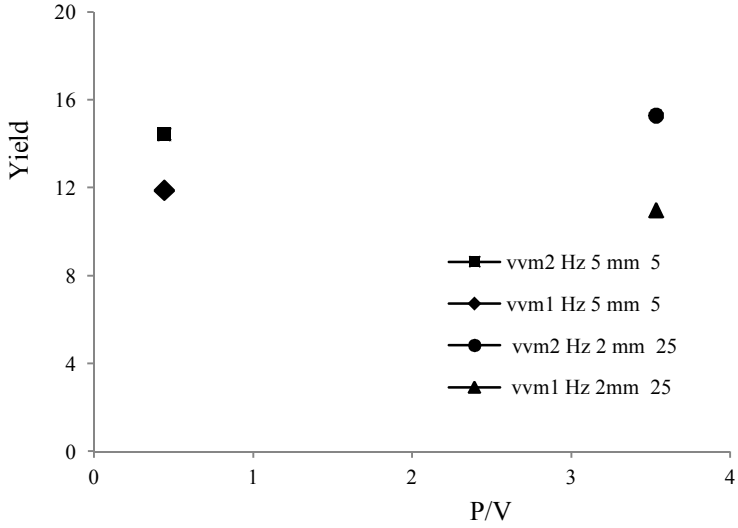


Figure 3.3: shows the power density of different agitation rates used in this study of STR vs. the yield after 96 hours.

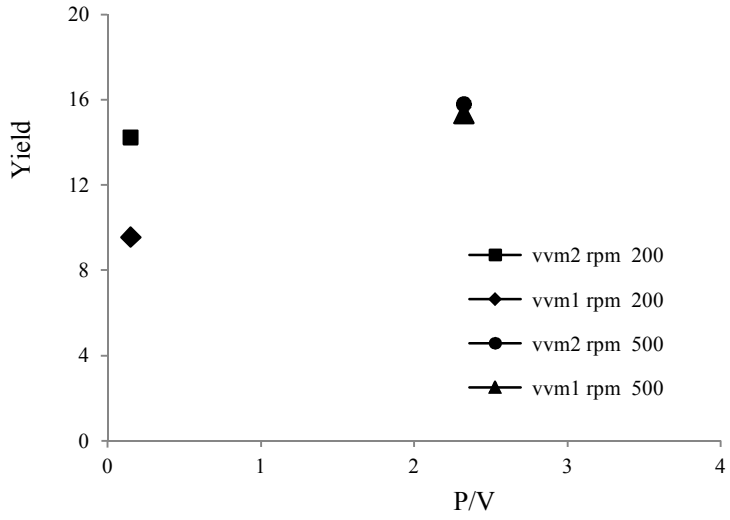


Figure 3.4: shows the power density of different agitation rates used in this study of STR vs. the yield after 96 hours.

Gaidhani (2004) studied the production processes of pullulan in the OBR and he compared his results with some studies done on the production of pullulan in the STR. The results showed that the production of pullulan in the OBR occurred much more rapidly with higher maximal concentration of pullulan than that in the STR. In addition, the quality of the product from the OBR is generally better, with a pullulan of higher molecular weight being elaborated. On the other hand the production of xanthan gum of the non-optimised OBR is at least equal to the traditional STR.

Two reasons could explain these differences in the production of these kinds of polysaccharides. Firstly, the morphology of both microorganisms is different. *X. campestris* is a single cell bacterium whereas *A. pullulans* is yeast-like fungus, therefore in the case of the STR, the baffles could trap the fungus during agitation which causes the accumulation of the microorganism on that area reducing the production of the polysaccharide but this does not occur in the OBR and therefore the production of pullulan is better in the OBR. Where in the case of xanthan, the baffles enhance the mixing; therefore, the production of xanthan in the STR is similar to that in the OBR. Secondly, xanthan has a high viscosity. It has been shown that the volume average shear rate in the OBR is generally of the order of 10-20 /s, which is significantly lower than that in the STR, where it is typically at least 100 /s or greater (Ni *et al.*, 1999,2000a; 2000b). This feature of global mixing, coupled with very low and uniform shear rate is beneficial to the pullulan production but less advantageous for xanthan production where shear sensitive cultures may be involved.

4. Conclusion

This work has demonstrated that the OBR is readilyadaptable to the cultivation of microorganisms for thesynthesis of useful products, such as high viscous polysaccharide, xanthan gum. Based on results of this study, power consumption of the production of high viscouspolysaccharides such as xanthan gum can be reduced in both OBR and STR by reducing the amplitude in OBR and agitation speed in STR with increasing the aeration rate.

Corresponding author
Ebtihaj Jambi
King Abdulaziz University, Girls section, Faculty of Science, Biochemistry Department
Email: ejjambi@kau.edu.sa

References

1. Becker, A., Katzen, F., Pühler, A., Ielpi, L. (1998). Xanthan gum biosynthesis and application: a biochemical/genetic perspective. *Applied microbiology and biotechnology,* 50 (2), 145-52.
2. Blanch, M., Legaz, M.-E., Vicente, C. (2008). Xanthan production by *Xanthomonasalbilineans*

infecting sugarcane stalks. *Journal of Plant Physiology,* 165 (4), 366-374.
3. Blanch, M., Legaz, M.-E., Vicente, C. (2008). Xanthan production by *Xanthomonasalbilineans* infecting sugarcane stalks. *Journal of Plant Physiology,* 165 (4), 366-374.
4. Byong, H. L. (1996). Fundamentals of food biotechnology. New York: VCH.
5. Gaidhani, H. K. (2004). An Experimental Investigation of Pullulan Fermentation in a Batch Oscillatory Baffled Fermenter. Edinburgh: Heriot-Watt University.
6. Gaidhani, H. K., McNeil, B., Ni, X. (2005). Fermentation of Pullulan Using an Oscillatory Baffled Fermenter. *Chemical Engineering Research and Design*, 83 (6), 640-645.
7. Garcia-Ochoa, F., Gómez Castro, E., Santos, V. E. (2000). Oxygen transfer and uptake rates during xanthan gum production. *Enzyme and Microbial Technology* , 27 (9), 680-690.
8. Jarman, T. R., Pace, G. W. (1984). Energy requirements for microbial exopolysaccharide synthesis. *Archives of Microbiology* , 137 (3), 231-235.
9. Lee, B. H. (1996). Fundamentals of food biotechnology. New York: VCH.
10. Marudova-Zsivanovits, M., Jilov, N., Gencheva, E. (2006). Rheological investigation of xanthan gum–chromium gelation and its relation to enhanced oil recovery. *Journal of Applied Polymer Science*, 103 (1), 160-166.
11. Moo-Young, M. (1985). Comprehensive biotechnology (pp. 1005-1041). Oxford: Pergamon Press .
12. Nasr, S., Soudi, M. R., Haghighi, M. (2007). Xanthan production by a native strain of X. campestris and evaluation of application in EOR. *Pakistan Journal of Biological Sciences*, 10 (17), 3010-3013.
13. Ni, X., Zhang, Y., Mustafa, I. (1999). Correlation of polymer particle size with droplet size in suspension polymerisation of methylmethacrylate in a batch oscillatory-baffled reactor. *Chemical Engineering Science* , 54 (6), 841-850.
14. Ni, X., Pereira, N. (2000a). Parameters affecting fluid dispersion in a continuous oscillatory baffled tube. *AIChE Journal* , 46 (1), 37–45.
15. Ni, X., Cosgrovea, J. A., Arnottb, A. D., Greatedb, C. A., Cumming, R. H. (2000b). On the measurement of strain rate in an oscillatory baffled column using particle image velocimetry. *Chemical Engineering Science* , 55 (16), 3195-3208.
16. Rosalam, S., England, R. (2006). Review of xanthan gum production from unmodified starches by Xanthomonas campestris. *Enzyme and Microbial Technology*, 39, 197–207.
17. Rushton, J. H., Costich, E. W., Everett, H. J. (1950). Power characteristics of mixing impellers. *Chemical Engineering Progress* , 46 (8), 395–476.
18. Sanderson, G. R. (1981). Applications of Xanthan gum. British Polymer Journal, 13 (2), 71-75.
19. Steinbuchel, A., Rhee, S. K. (2005). Polysaccharides and Polyamides in the Food Industry. Weinheim: Wiley.
20. Yoshida, T., Tanner, R. D. (1993). Bioproducts and bioprocess (Vol. 2). Berlin: Springer-Verlag.

11/121/2013