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ABSTRACTThe present report aims to shed light on theoptimization of the solid/liquid separation using centrifugation. This involvesboth type of centrifuge used and operation characteristics, such as flow rate. Moreover, it investigates the centrifuge operation upon integration withfollowing processing steps, such as filtration and chromatography. For this reason, the disk stack centrifuge was used toclarify yeast homogenate, in different flow rates. The clarification wasestimated based upon the optical density values obtained for the supernatantafter the centrifugation.

Furthermore, evaluation of the centrifugation wascarried out through filtration experiments for each flow rate. This was done byobtaining the flow rate of the supernatant when passing it through the filter. The obtained results were compared to analogous experiments for yeast and E. coli. The experiments showed better clarification for lowflow rates. The highest clarification achieved was 99.

31% and was obtained whenthe flow rate was the lowest studied, and equal to 0. 6 L/min. The filtrationresults showed decreasing filtration flow rate for increasing flow rate incentrifugation. In conclusion, this study showed decreasingclarification for increasing flow rate, but showed that other aspects areimportant as well, especially when it is integrated is a multistep process.(200 words)RESULTSThe opticaldensity of the supernatant for each flow rate is plotted versus time in Figure1. Figure 1: Optical density of the supernatant measuredeach minute over 10 minutes time, for each flow rate studied.

The opticaldensity values reach a steady state, different for each flow rate used. Thesteady state optical densities observed for each flow rate, as well as the timeneeded to achieve that value are presented in table 1. Table 1: Steady state optical densities and timerequired to reach these values for all of the flow rates studied. The number ofbowl volumes of feed until steady state are also presented, showing highervolume requirements for higher flow rates. Flow rate (L/min) Steady state OD value Time required to achieve steady state (mins) Number of bowl volumes of feed required to reach steady state 0. 6 0. 105 6 3.

6 0. 875 0. 116 7 6. 1 1. 2 0. 146 8 9.

6 1. 5 0. 147 8 12 2. 2 0. 171 7 15. 4 Figure 2shows the dependence of the steady state OD values of the supernatant on theflow rate used. Figure 2: Steady state ODs measured for each feed flowrate. In the diagram it seems that the higher the flow rate, the higher thesteady state OD value.

The dataacquired suggest a quite good quality of the experiments, as they showed theexpected. That is the lower ODs observed were for the lower flow rates used. The number of bowl volumes of feed required to reach thesteady state clarification are summarized in table 1, for every flow ratestudied. This number is indicative of the flow pattern in the centrifuge andthus it can be used to predict the likely flow pattern in the centrifuge.

Onecentrifuge volume required to be processed until steady state is reached, isindicative of plug flow, i. e. ideal flow without mixing. On the other side, forthe fluid to be well mixed the volume processed until steady state should befive or more bowl volumes. Since all flow rates investigated required more than 3. 6 bowl volumes of feed to be processed to reach steady state, in noneof the cases there is plug flow.

Instead, almost all of them are well mixed. Whenthe flow rate was 0. 6 L/min 3. 6 bowl volumes of feed were processed untilsteady state was reached.

In this case the flow pattern is neither plug flownor well mixed, but something in between. The higher flow rates, i. e. 0. 875L/min, 1. 2 L/min, 1. 5 L/min and 2.

2 L/min led to the processing of 6. 1, 9. 6, 12and 15. 4 bowl volumes of feed until steady state, which is indicative of a wellmixed flow pattern in the centrifuge.

The clarifications achieved for each one of the flowrates, after steady state was reached, are provided in table 2. Table 2: Clarification values observed for the different flow rates studied. Theincrease in the flow rate leads to decrease in the clarification achieved. TheQ/?(m/s) values are presented as well, following an increasing path when flow rateis increased. Flow rate (L/min) Clarification % Q/? (m/s) 0.

6 (0. 1 L/s) 99. 31 0. 00019 0. 875 (0. 15 L/s) 98. 98 0.

00028 1. 2 (0. 2 L/s) 98. 07 0. 00038 1. 5 (0. 25 L/s) 98.

04 0. 00048 2. 2 (0. 37 L/s) 97. 31 0. 00070 The ? factorfor the centrifuge used in the present experiments, which was the CSA-1 diskstack centrifuge, was calculated to be equal to 525. 0 m2, and the Q/? ratios for each flow rate

are presented in table 2. Figure 3 shows theclarification plotted versus Q/? Figure 3: Clarification plotted versus Q/?.

Higher flow rate, Q leads to higher Q/?. From the diagram it seems that there is aclear decrease of the clarification with decreasing Q/?. In Figure 3 there is a clear correlation of the Q/? with the clarification. Filtration experiments were also carried out in orderto evaluate the performance of the centrifugation for each flow rate. For theevaluation, the volume of filtrate was measured after one minute of filtration.

Then, the flow rate during filtration was calculated. These values aresummarized in table 3. Table 3: Filtration rates for the different flowrates that were used in the centrifugation. The filtration rate of thehomogenate feed and the well spun were evaluated as well and used asreferences.

Higher centrifugation flow rates led to lower filtration rates, meaning that higher throughput led to lower performance of the centrifuge. Sample Filtration volume in 1 min (mL) Filtration rate (L/hr) Homogenate feed 9 0, 54 Well spun 11 0, 66 centrifugation flow rate (L/h) Filtration volume in 1 min (mL) Filtration rate (L/hr) 36 12 0. 72 52. 5 12 0. 72 72 11 0. 66 90 9 0. 54 132 10 0. 6 Table 3shows a quite decreasing filtration rate for increasing flow rate during centrifugation, which is result of the poorer performance of the centrifuge for higher flowrates.

DISCUSSIONi) The flow sheet from fermentation to the firstchromatography column is shown in Figure 4. Figure4: Flowsheet for the extraction of intracellular protein from yeast. After thefermentation of the yeast cells, the broth is centrifuged to separate the cellsfrom the liquid phase, cells are then resuspended. Cell disruption is achievedthrough high pressure homogenization and the liquid is centrifuged for a secondtime in order to remove yeast cells debris. The product is in the supernatant, which is later filtrated in order to be prepared for the first chromatographystep.

ii) Centrifugation and filtration are two major stepsbefore chromatography, as they remove the solids that would lead to fouling ofthe chromatography column. In the case of the yeast cells homogenate, the feedinserted into the centrifuge is a mixture of large and smaller cell debris, aswell as proteins in a range of sizes. In order for the product protein to be extracted, it has to be removed out of a pool with solids/proteins which have a comparablesize with the target protein. Therefore larger solids, i. e. membranes, largeprotein aggregates and organelles, have to be removed first. This occurs mostlyduring centrifugation. These attributes render centrifugation priorchromatography an essential step.

iii) Some of the most important aspects of the disk stack, multichamber bowl, tubular bowl, solid bowl and scroll decanter centrifuges arepresented in table 4. Table 4: Advantagesand disadvantages of the disk stack, multichamber bowl, tubular bowl, solid bowl and scroll decanter centrifuges. Themost important characteristics of a centrifuge are the settling area and theoperating rotatioal speed and flow rate, as these are the factors thatinfluence the quality of roduct, i. e. clarity of the supernatant, and thethroughput of the centrifuge as well. 1-7 Centrifuge type Advantages Disadvantages Disk stack · Large equivalent settling area ?, meaning large processing area ·High centrifugation speed available · Easy to operate · Small footprint · Large processing volumes · Operation at high flow rates feasible · ? nly for small solid content in feed Multichamber bowl · High centrifugation speed available · Small settling area · Operates at low flow rates Tubular bowl · Processing both liquid/liquid and solid/liquid separations · Possible foaming Solid bowl centrifuge · Higher centrifugation speed available · Operates at low flow rates Scroll decanter centrifuge · Continuous operation available · Short cleaning time · Complex iv) Steady state opticaldensities observed for the different flow rates studied. Figure 2 shows that the lower the flow rate used thebetter the clarification achieved.

This means fewer solids in the supernatantand thus lower OD values. For example, for the flow rates 0. 6 L/min and 0.

875L/min, which were the lowest flow rates studied, the steady state OD valueswere 0. 105 and 0. 116 correspondingly. On the other hand, for the higher flowrates 1. 2 L/min, 1. 5 L/min and 2. 2 L/min these values were 0.

146, 0. 147 and0. 171, which are higher than those observed with the low flow rates. Thesefindings are in line with the expected pattern of increasing OD values withincreasing flow rate, as less yeast debris are removed and ODs is proportionalto the cell debris in the liquid. Clarification values observed for the different flowrates studiedFor the lowest flowrate 0. 6 L/min, the clarification percentage was 99. 31%, which was the highestclarification observed. The 0.

875 L/min, 1. 2 L/min and 1. 5 L/min followed with98. 98%, 98. 07% and 98. 04% clarification correspondingly, while the highest flowrate, i. e. 2.

2 L/min resulted in 97. 31% clarification, the lowest among all. Asexplained earlier in this report, this is due to the fact that the low flowrates enable the centrifuge operate in the best way, from the aspect of solidremoval. Clarificationplotted versus Q/? Table 2 shows that higher flow rates exhibited higherQ/? values, as ? is constant for a specific centrifuge. So forexample, when the flow rate was 0. 6 L/min, Q/? was 19*10-8 m/s, while for the 0. 875 L/minflow rate, the same value was 28*10-8 m/s.

For operation at higher flow rate the Q/? values were higher. In moredetail, these where 38*10-8 m/s, 48*10-8 m/s and70*10-8 m/s for the 1. 2L/min, 1. 5 L/min and 2. 2 L/min correspondingly. Effect of Q/? on clarificationThe values obtainedfor the clarification, as presented in figure 3, show that clarification isinversely proportional to the Q/?. The highest clarification that was achieved was 99.

31 %, and wasobserved when Q/? was equal to 19*108 m/s. the lower clarification values98. 98 %, 98. 07 %, and 98. 04 % follow, for the Q/? values 28*108 m/s, 38*108 m/s and 48*108 m/s correspondingly. The lowest clarificationcorresponded to the highest Q/? value, 70*108 m/s, and was found to be 97. 31%. Filtration experimentsTable 3 shows that high flow rates for centrifugationresulted in lower clarification levels and higher ODs values, both indicatinghigher presence of solids in the liquid.

These solids are the key to the lowerflow rates observed during filtration, as these tend to block the membrane. Similarly, lower flow rates led to lower presence of cell debris n the materialand therefore the liquid was able to move through the filter pores with moreease. Specifically, the 36 L/hr and 52.

5 L/h flow rates of centrifugationresulted in the same filtration rate 0. 72 L/h. The 72 L/h flow rate gave aliquid which passed through the membrane with 0. 66 L/h, while the higher flowrates 90 L/h and 132 L/h were found to correspond to 0.

54 L/h and 0. 6 L/hcorrespondingly. These values are not totally in line with the expectedpattern, such as the 90 L/h and 132 L/h flow rates where the higher flow ratein centrifugation gave higher filtration flow rate. This deviation may beattributed to human errors in handling during filtration, such as membraneplacement or time measurement. Other affecting factors could the fact thatthere was only one filtration experiment for each sample, as well as the approximatemeasurement of the volume filtrated in 1 min.

In thecase of an integrated platform of centrifugation and filtration as apreparation step for a chromatography column, the size of the filter woulddepend on the flow rate of the incoming liquid in the centrifuge. In order fora high flow rate to be used during centrifugation, a larger size of filterwould be required to ensure the solid removal without blockage of the membrane, as there would be more remaining solids in the liquid for high flow rates. Inthis way, the whole volume of liquid will be processed during filtration and bewell prepared for chromatography. It is essential that solid removal issuccessful prior to the chromatography step, as in the opposite case the columnwould block.

In the same way, in the case of lower centrifugation flow ratesused, a smaller filter would be sufficient for the preparation of the feed forthe chromatography, as the solids would be less, as indicated by the previousdata presented. v) My clarification values are similar to the clarificationvalues obtained for different Q/?, as reported by Bracewell et al (2008) for yeast homogenates. The clarificationsachieved ranged from low percentages to 99% for the studied operatingconditions. On the other hand the reported values for E. coli homogenates, as published by both Li et al (2013) and Chatel et al (2014) are much differentthan those of yeast homogenates.

Both studies showed a lower clarificationachieved for E. coli homogenates. Forexample, Chatel et al (2014) found that for Q/? 4*10-8 less than 60% of the solids wereremoved. Same, Li et al (2013) found that only 30% of the solids were removedfor the same Q/?, while the clarification achieved for yeast homogenates was more than 97% forevery Q/?. Though, in all studies including the present report, the clarification had a decreasing pattern for increasing Q/? values. In fact, the above studies showed that the level of clarification of E.

coli homogenates could reach theclarification levels of the yeast homogenates for much lower operating Q/?, such asless than10-9 value for Q/?. 8-10 Solid removalwith combination of methodsThe use of an integrated platform with a series ofsteps enables further optimization of the process as a whole, to give betterresults than each step alone would give. For example, the opportunity ofchoosing a better throughput for the centrifugation step is given, as thefollowing filtration step will make up or the lower quality of separationduring the centrifugation step. To study this, I would conduct a series ofexperiments using different centrifuge types operating at different flow ratesin combination with different filtration steps. The difference in thefiltration step could be the size of the filter, the size of the pores, as wellas the pump forcing the liquid to flow. The process could be evaluated on the basisof output material quality and total operation time. Investigation of the finalmaterial could be done through a following chromatography step, e.

g. to measurethe fouling of the chromatography column, or measurement of its opticaldensity. REFERENCES1.

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(2008), Impact of clarification strategy on chromatographicseparations: Pre-processing of cell homogenates. Biotechnol. Bioeng., 100: 941–949. doi: 10. 1002/bit. 21823 APPENDIXEstimation of the steady state OD valuesThese valueswere estimated based on Figure 1.

These correspond to the OD value where no changesin OD are observed beyond that point. For example, for the 0. 6 L/min flow rate, the OD seems to be stabilized in the 0. 105 value, as the next values are closeto 0. 105 and 0. 105 was the OD of the supernatant for a second time, after 9minutes of centrifugation. The methodology used is clearer for the flow rate of1. 2 L/min.

In this case, OD rises with time but stabilized at 0. 146 which isthe steady state OD value. Calculation of bowl volumes of feed For thecalculation of this volume it is essential to know the time required to reachsteady state. This time is presented in table 4 as well, together with the ODvalues for each time interval.

By multiplying this time interval with the flowrate, the calculated value is the total volume of feed needed to be processeduntil steady state. The number of centrifuge bowls that this volume is equalto, is (volume of feed processed)/(centrifuge volume). Therefore, the number ofbowl volumes of feed that are required to be processed until steady state OD isequal to (Q*ts)/VB, Where Qis the flow rate, tsis the time required until steady state and, VB isthe total volume of the centrifuge. Forexample, for the calculation of the bowl volumes of feed required to reach ODsfor the 1. 2 L/min it is: Q= 1. 2L/mints= 8min. The totalcentrifuge volume VB is 1.

0 L for the Pathfinder disk stackcentrifuge and therefore it is: Bowlvolumes of feed = (1. 2 L/min)*(8 min)/(1. 0 L) = 9. 6The exactsame methodology was used for all flow rates under investigation. Calculation of clarificationThe clarificationpercentage for each flow rate was used via the formula C =(ODf-

ODs)/(ODf-ODw)*100% (1), Where ODfis the OD value of the feed, ODs theOD value of the supernatant of the centrifuged feed under certain flow rate, andODw isthe OD value of the supernatant after high speed centrifugation for long time-this is practically the most clarification that can be achieved throughcentrifugation. Theclarification was calculated in the same way for every flow rate studied. Anexample calculation is that for the 0.

6 L/min flow rate. The ODfwas measured to be equal to 3. 377, while the ODw was 0.

082. Also, the steadystate OD value for the 0. 6 L/min was estimated to be 0. 105. So, from (1) it isC = (3. 377-0.

105)/(3. 377-0. 082)*100= 99. 312 % or 99. 31%. Calculation of the ? factorTheequivalent settling area ? of the Pathfinder disk stack centrifuge used, the CSA-1, is given by thefollowing equation: ?= (2*?*F)/3*(z/g)*? 2*cot?*(Ro3– R13)*Cds (2), Where Ro is theouter radiusR1 is theinner disc radiusZ is thenumber of discs in the stack? is the half disc angleF, thecorrection factor for area occupied by caulks, andCds isthe calibration factor for non-ideal flow. For the centrfuge used these valuesare known to be as: Ro= 0.

055 m R1= 0. 0261 m z= 45 discks?= 38. 5 degreesF= 0. 9N= 9800 rpmCds= 0. 4The operating speed of the centrifugeis 9800 rpm, which is equivalent to (9800 rpm)/(60 s*min-1) = 163.

33rps. Therefore ?, which is equal to 2*?*?, is ? = 2*?*? = 2*3. 142*163. 33 s-1 = 1026. 387 s-1.

Since cot? = 1/tan? = 1/tan38. 5 = 1/1. 031= 0, 969 it is cot? = 0, 969Therefore (2) becomes?= (2*3. 142*0. 9)/3*45/(9. 81m*s-2)*(1026. 387s-1)2*cot(38.

5)*(0. 055 m)3– (0. 0261 m)3*0. 4= 524. 967 m2. Calculation of Q/? valuesSinceboth the flow rate Q values and ? are known, Q/? values were calculated easily.

The following is an example calculation. For Q = 0. 6 L/min = (0. 6 L/min)/(60 sec*min-1) = 0. 1 L/s or 0. 1*10-3m3s-1. So, it isQ/?= 0.

1*10-3 m3s-1/ 524. 967 m2 = 0. 00019*10-3ms-1.