Centrifugal Analogs for Tangential Flow Microfiltration

in Recombinant Cell Processing

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OBJECTIVES

Provide an overview of centrifugal analogs to tangential flow microfiltration and their application to solids washing applications in recombinant cell processing

- Overview of Solids Washing Operations
- Development of Process Model
- Presentation of Case Study

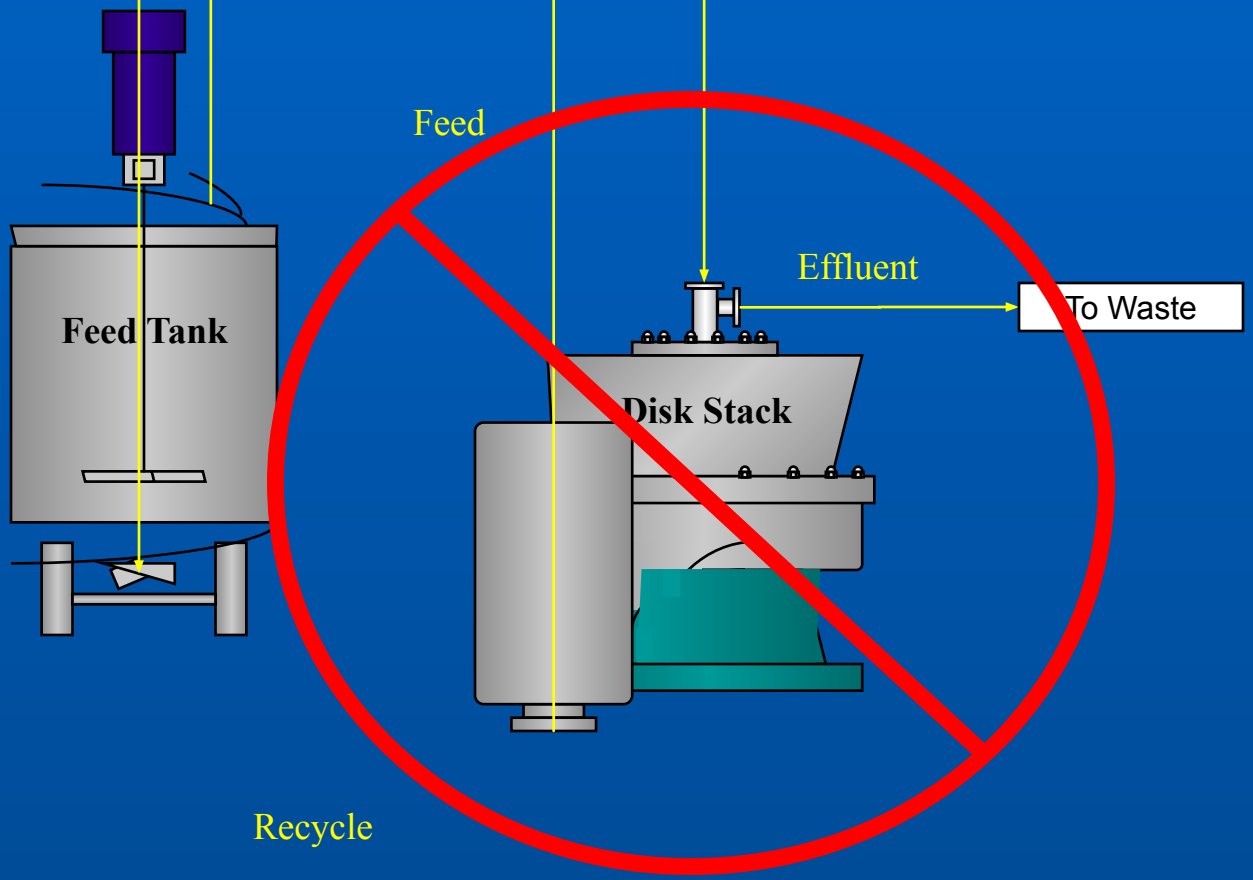
Overview of Solids Washing Operations in Microbial Cell Processing

- Designed to Remove Soluble Components
  - Fermentation Media Components
  - Intracellular Host Cell Protein Impurities
  - Harvest Soluble Product From Cellular Lysates

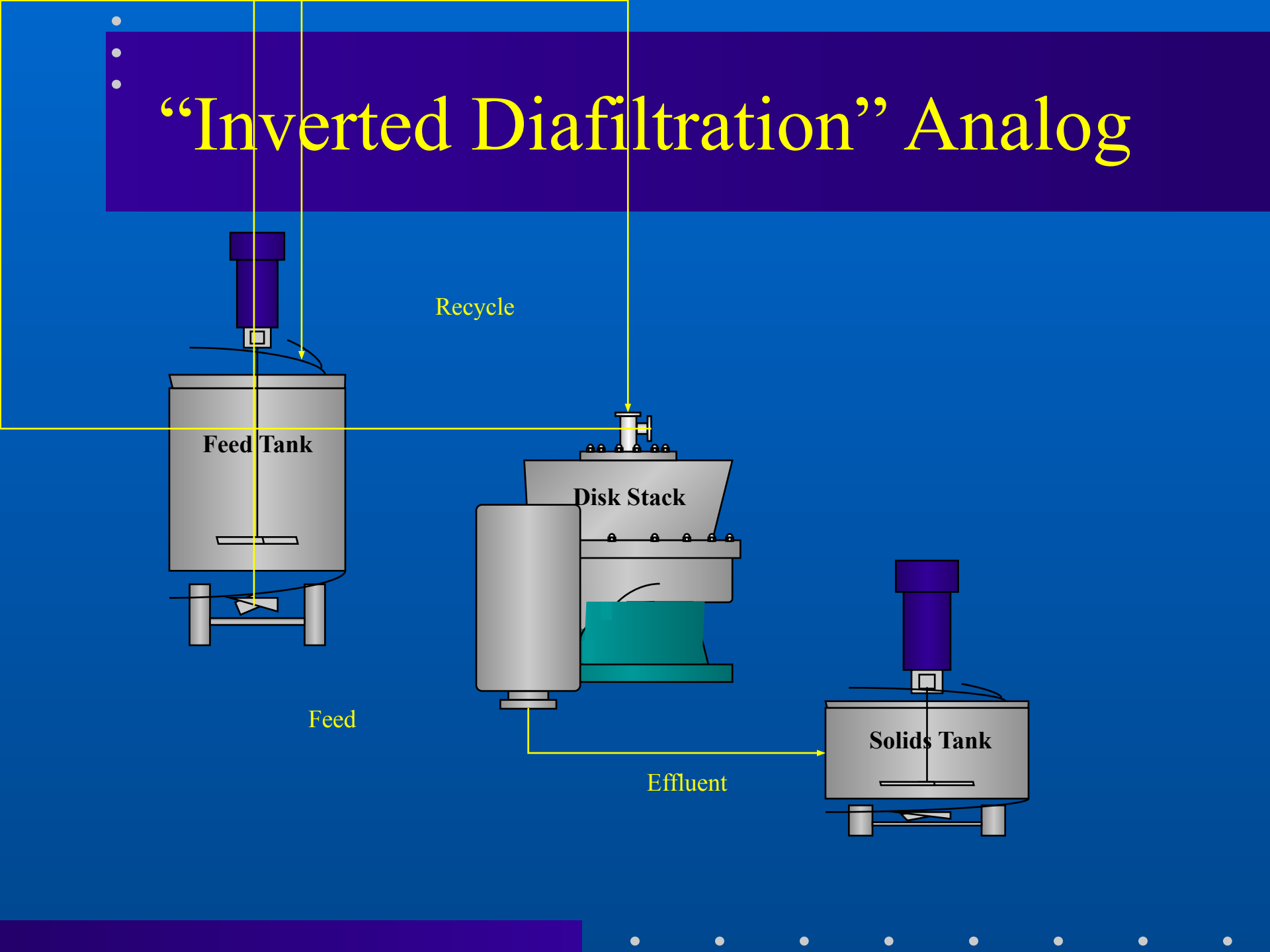
Overview of Solids Washing Operations in Microbial Cell Processing

- Two Types:
  - Concentration Operations
  - Buffer Exchange Operations
- Unit Operations for Solids Washing
  - Sequential Centrifugation
  - Tangential Flow Microfiltration

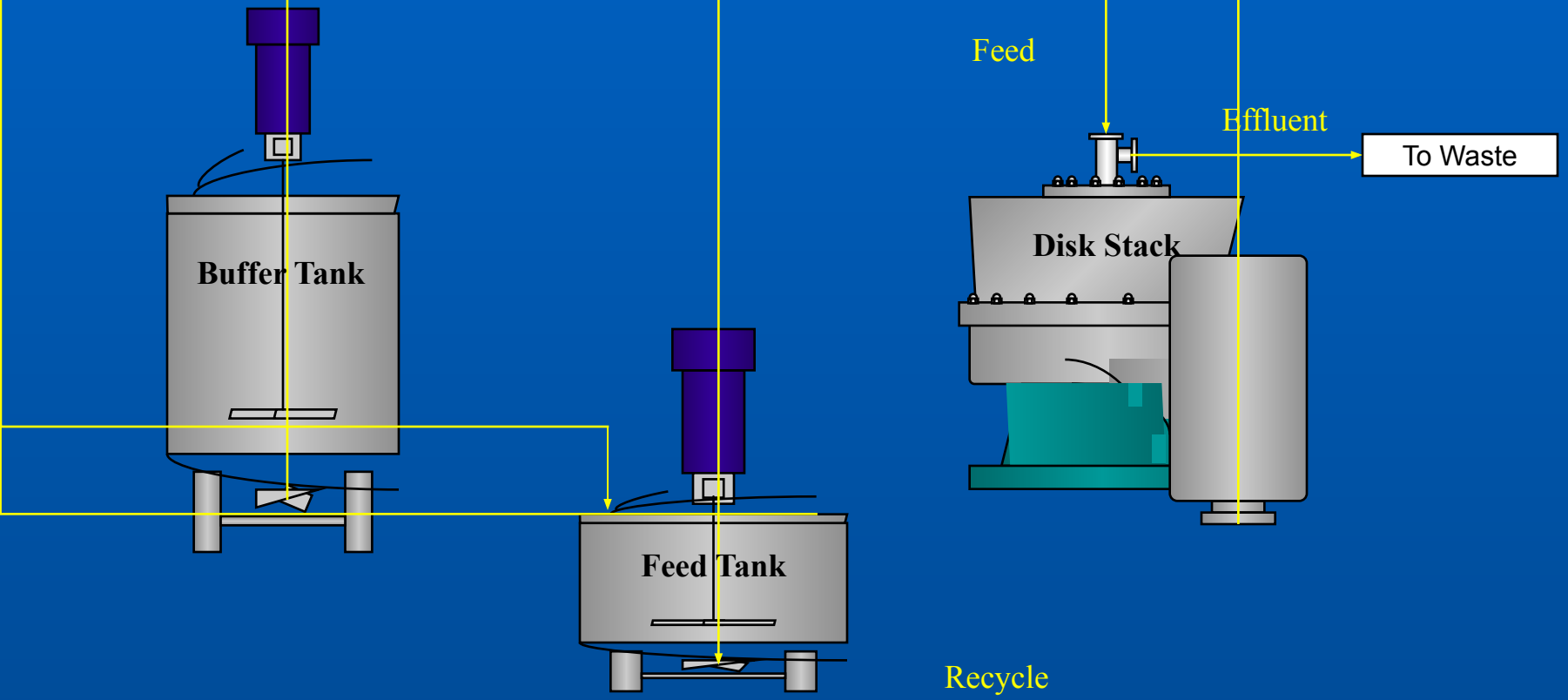
An Exact Analog for Concentration



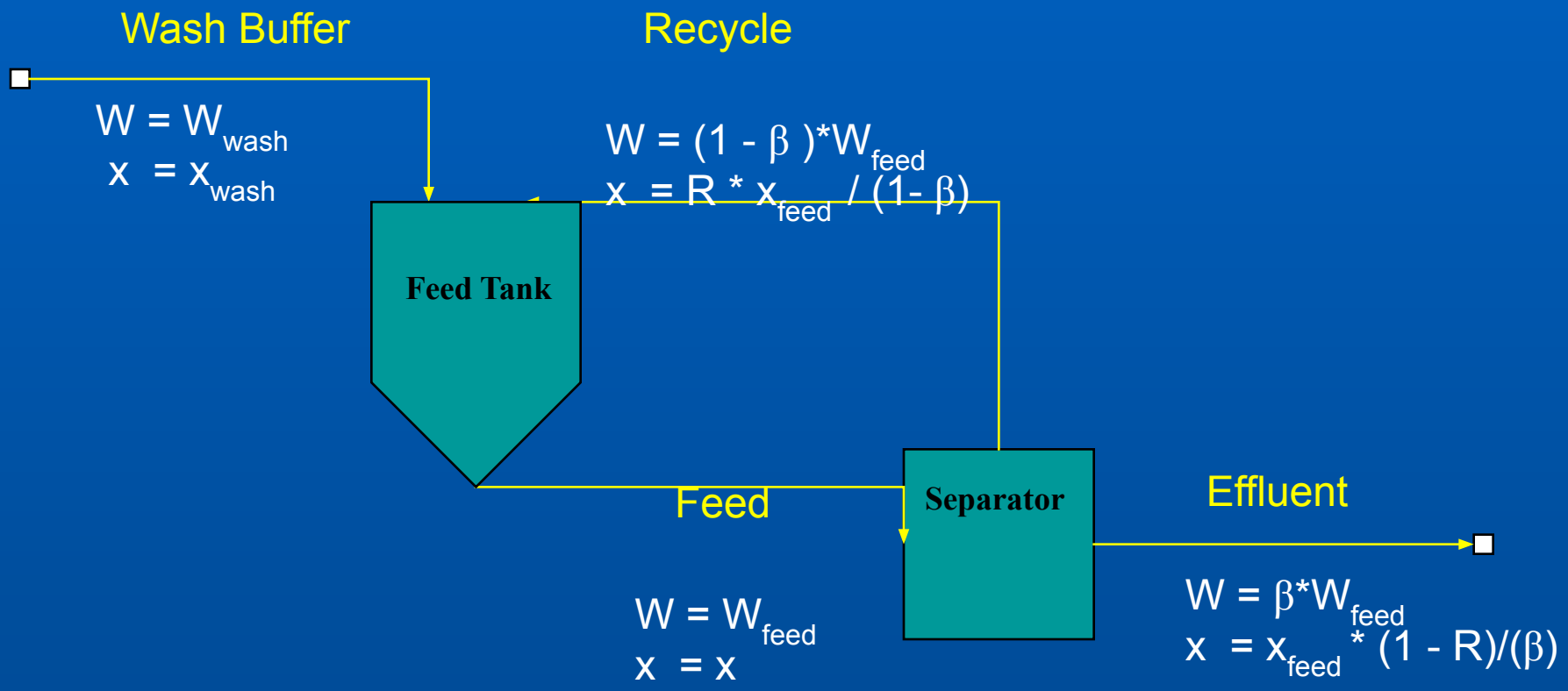
“Inverted Diafiltration” Analog



Diafiltration Analog



Materials Balance Model for Comparison of Recycle Cell Processing Operations



The Rejection Coefficient (R)

TFF Microfiltration

$$R = \frac{1}{1 + \exp\left(\frac{D - D_c}{w}\right)}$$

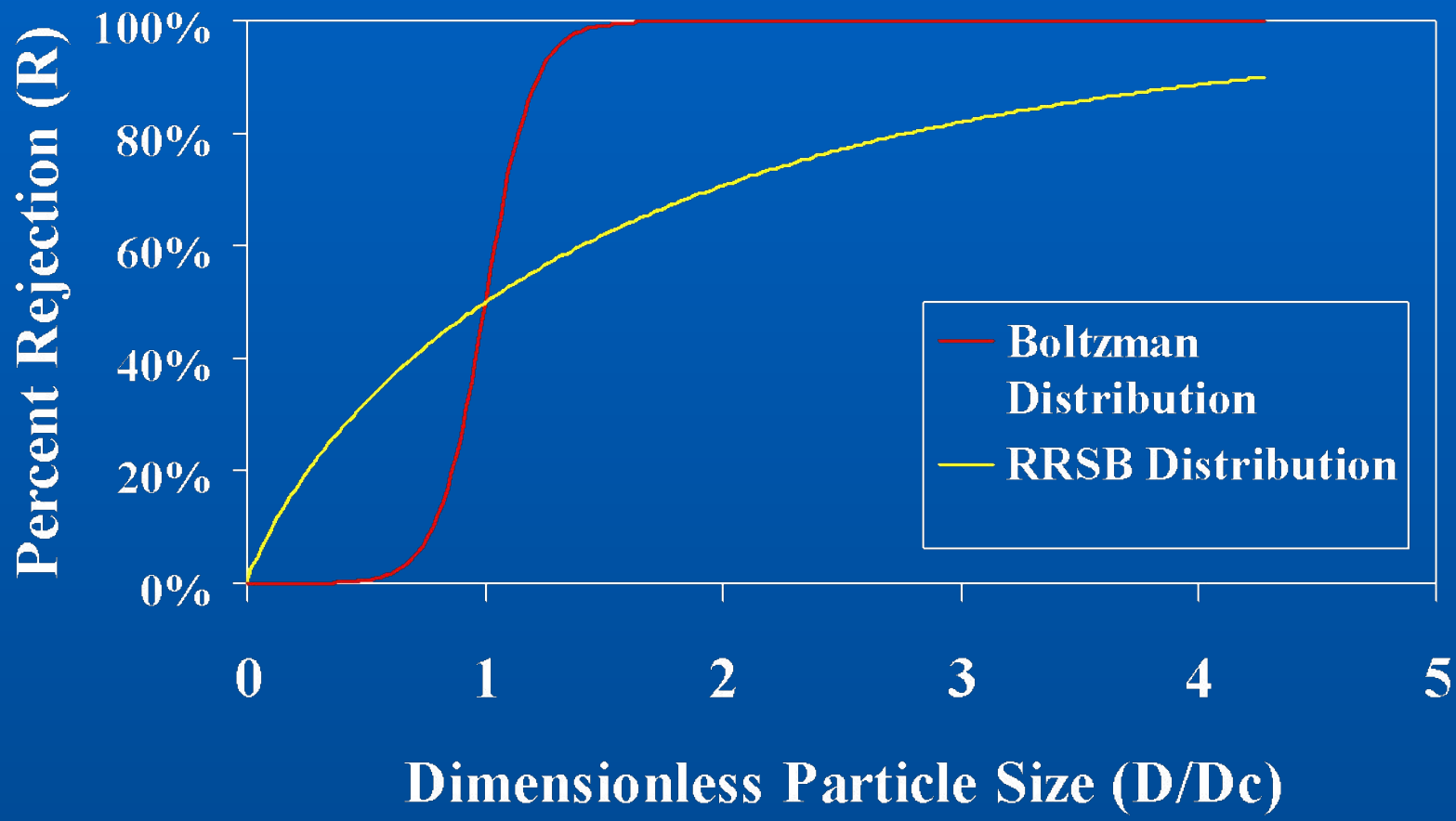
Boltzman Distribution

Disk Stack Centrifuge

$$R = 1 - \exp\left(-k \left(\frac{D}{D_c} \right)^n (1 - x)^{4.8} \right)$$

RRSB Distribution
[Mannweiler 1990]

A Comparison of Typical Grade Efficiencies



Conversion (β)

TFF Microfiltration

$$\beta = 0.024\left(\frac{D^4}{L}\right)^{1/3}\frac{\gamma_w A\rho}{W}Ln\left(\frac{.58}{x}\right)$$

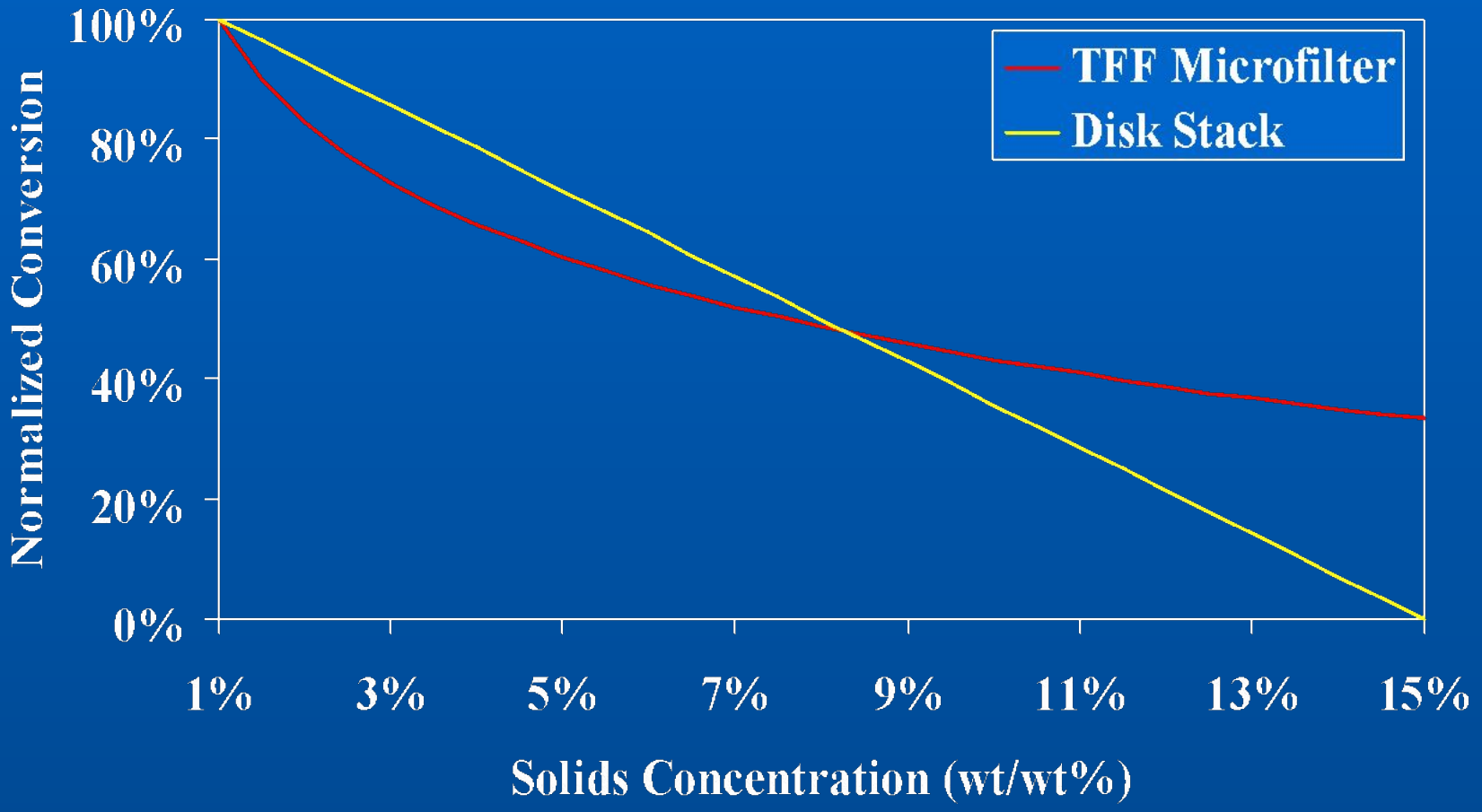
Shear Diffusion Theory
[Zydney & Colton 1985]

Disk Stack Centrifuge

$$\beta = 1 - R\frac{x}{x_{shot}}$$

Specified by
Shot Frequency

The Effect of Concentration on Conversion



Effect of Shot Frequency on the Processing Time

- Bowl Desludging not instantaneous
  - typically 0.5 - 3 minutes

- Total Number of Bowl Shots

$$N = \frac{W_{recirc} t}{M_{shot}} \frac{Rx}{(x_{shot} - x)}$$

During Exchange Operations

$$N = \frac{M_o}{M_{shot}} \frac{Fx_o}{(x_{shot} - x_o)}$$

During "Inverse DF" Operations

Case Study

- GOAL

  Removal of 99% of the media components from a bacterial suspension by concentration and buffer exchange.

- CONSTRAINTS

  Cell wash must yield at least 90% recovery of solids.

Case Study - Parameters

SUSPENSION | MEMBRANE | CENTRIFUGE

TYPICAL BACTERIAL FERMENTATION BROTH

Batch Size:	100 kg
Initial Solids Concentration:	1% (wt/wt)
Fluid Density:	1.05 g/cm^3
Fluid Viscosity	1.5 cP
Solids Diameter	1 micron
Solids Density	1.3 g/ cm^3

Case Study - Parameters

SUSPENSION | **MEMBRANE** | CENTRIFUGE

COMMERCIALLY AVAILABLE FLAT SHEET MICROFILTER

Area Per Module:	9 ft^2
Crossflow Rate	1.5 m/sec
Length Per Module:	33 cm
Width:	15.7 cm
Channel Height:	0.7 mm
Channels per Module:	11

Case Study - Parameters

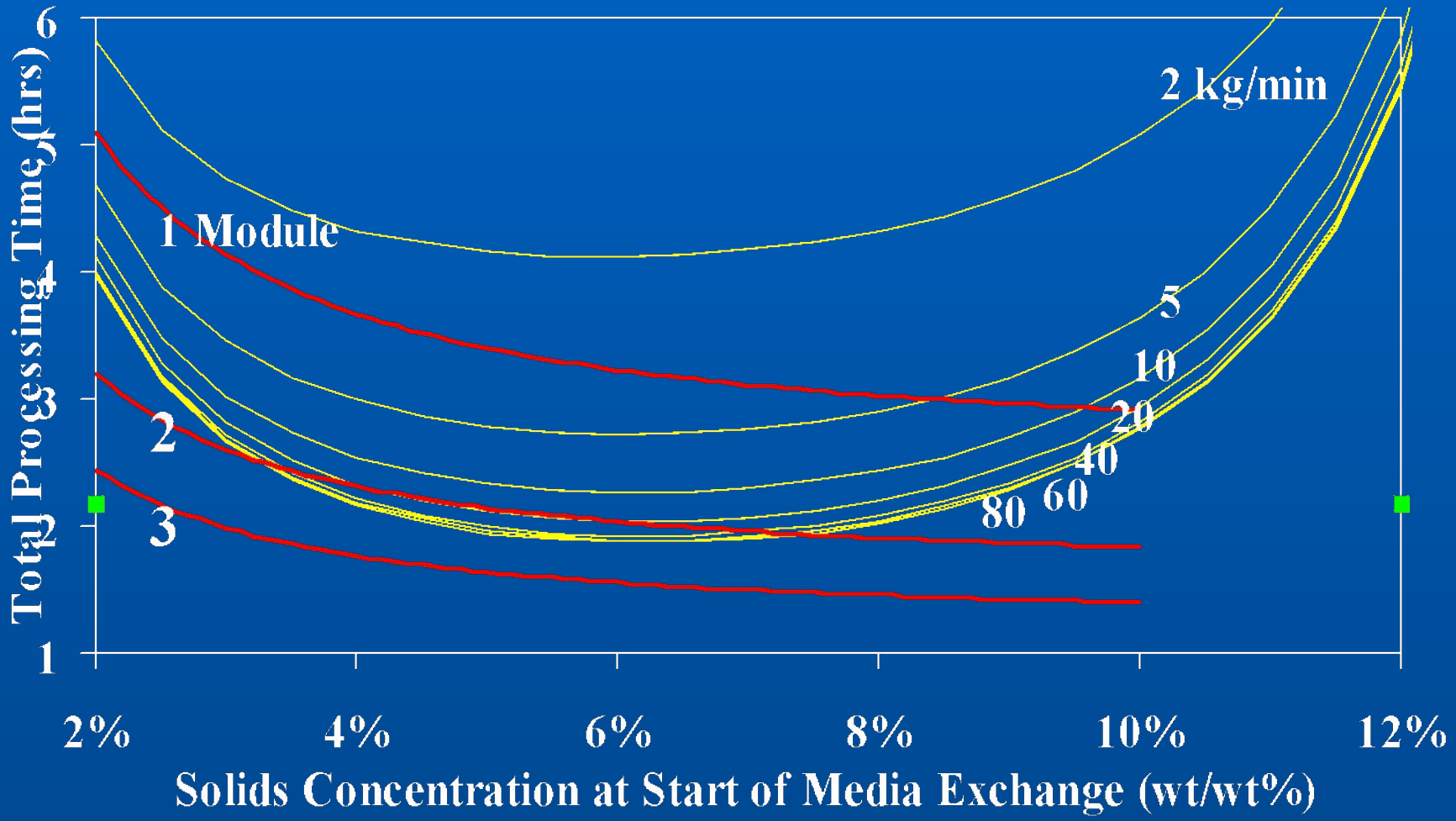
SUSPENSION | MEMBRANE | **CENTRIFUG E**

COMMERCIALLY AVAILABLE DISK STACK CENTRIFUGE

Sigma Factor	9100 m^2
Hydraulic Capacity	80 LPM
RRSB Parameters	k = 0.129
	n= 0.828
Shot Size	1.5kg
Bowl Shot “dead time”	60 sec

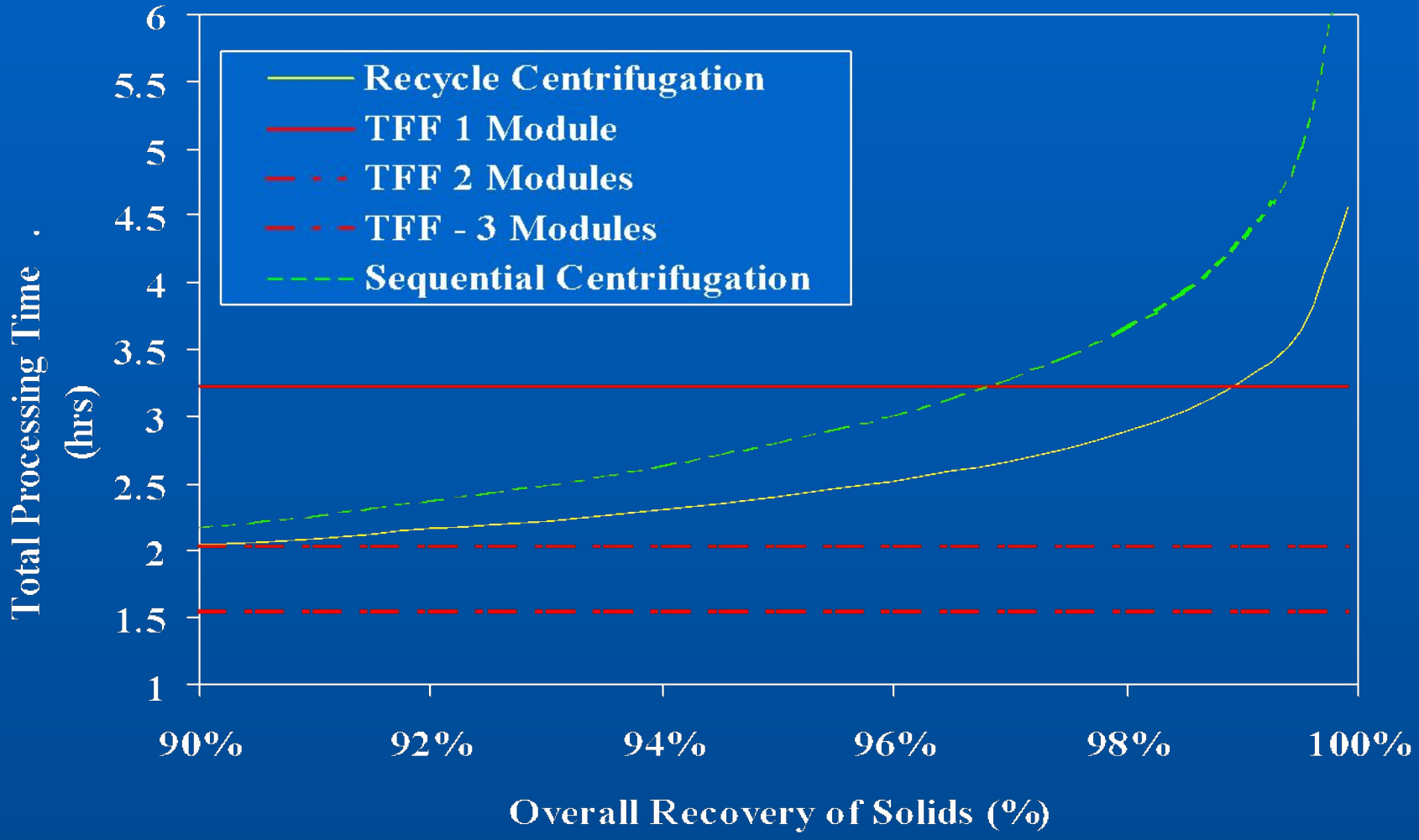
Case Study

Total Processing Time as a Function of Extent of Preconcentration



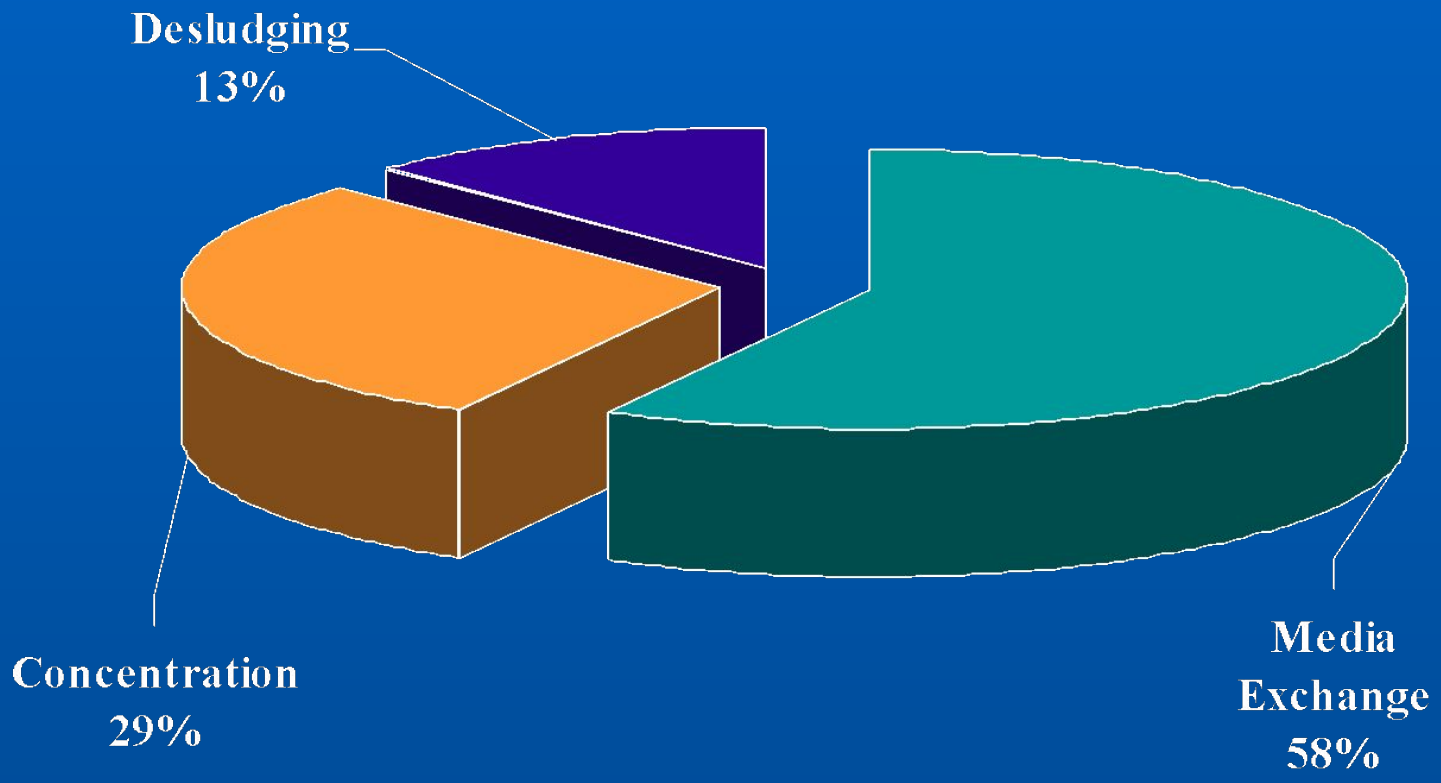
Case Study

Processing Time as a Function of Overall Recovery of Cells



Case Study

Breakdown of Processing Time



Conclusions

- Recycle Centrifugation has competitive cycle times
- Cycle times exhibit a minima at the optimal preconcentration mass fraction.
  - Shot Frequency
  - Batch Size
  - Initial Solids Concentration

Conclusions

- Which unit is best suited for the process depends on specific process parameters
  - –Particle Size Distribution History
  - –Classification
  - –Extent of Concentration
  - –Batch Size
  - –The Process Equipment
  - –Process Economics

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