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Generic flowsheet model for early inventory estimates of industrial microbial processes. II. Downstream processing

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ABSTRACT

To ensure optimal process flowsheet selection it is valuable to conduct environmental and economic comparisons at an early stage of technology selection and process design. However, the data that is needed to perform these studies are not available at this stage of process development. This is also true for bioprocess systems. To overcome the lack of data, the CeBER (Centre for Bioprocess Engineering Research, University of Cape Town) Bioprocess Modeller was developed to provide material and energy values for industrial microbial processes.

This paper presents the downstream processing portion of this flowsheet. The model allows for solid–liquid separation, cell disruption, concentration and formulation units as required. The model allows section of appropriate downstream processing units include, amongst others, centrifugation, filtration, precipitation and freeze-drying. At each downstream processing stage, non-reacting and reacting chemicals can be added. The model provides both a material inventory as well as the calculation of the energy input required and waste heat generated.

Additionally, the model includes a database of values (including constants, operating conditions and others), drawn from various industrial norms and academic sources. Should specific information not be known, the model selects the most appropriate values based on other decisions made through the model.

1. Introduction

For microbial bioprocesses, it is accepted that the relative economic importance of the production and downstream stages is highly dependent on the product value and purity required. The relative importance of these stages with respect to environmental burden has yet to be addressed. Process flexibility decreases as the level of definition increases in the system; hence it is valuable to be able to analyse the potential for combined process sustainability as well as technical and environmental feasibility during the early stages of design. To achieve this, estimates of the material and energy inventories are required.

In order to obtain easy access to accurate material and energy inventory data for the modelling of industrial bioprocesses at the early stages of process design, a Microsoft®

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Table 1 – Literature review of commonly used downstream process units.

Product (Microorganism)	Separation[a]	Concentration and Purification[b]	Formulation[c]	Reference
Anthracycline antibiotics (*Streptomyces* sp., *E. coli*)	Broth filtration, solvent extraction ($CHCl_3$, butanol) centrifugation	Precipitation (dissolved with *n*-butanol. Acetone added)	Freeze drying	(Flickinger, 1985)
Cephalosporins	Filtration (rotary drum), solvent extraction (methyl isobutyl ketone)	Precipitation (acetone added), adsorption (Activated carbon, non-polar resins), enzyme treatment		(Smith, 1985)
Cephamycin	Solvent extraction (acetone (aq.), 50% v/v.)	Evaporation		(Omstead et al., 1985)
Citric Acid (*Aspergillus niger*, yeasts)	Filtration, solvent extraction (butan-2-ol, tributyl phosphate), centrifugation	Precipitation (calcium citrate)		(Milsom and Meers, 1985a)
Glycerol (*Aspergillus niger*)	Broth filtration, centrifugation	Precipitation, evaporation, ion exchange	Spray drying	(Milsom and Meers, 1985b)
Itaconic acid (*Aspergillus terreus*)	Broth filtration, centrifugation	Precipitation, evaporation		(Milsom and Meers, 1985b)
Lactic acid (*Lactobacillus* sp.)	Filtration, solvent extraction, distillation	Precipitation, evaporation		(Vickroy, 1985)
Lincomycin (*Streptomyces* sp.)	Filtration (4.0% filter aid before filtration. Water washed), solvent extraction (activated carbon, *n*-butanol)	Ion exchange (cationic resins), partition chromatography (cyclohexane, methyl ethyl ketone)	Freeze drying	(Gonzales and Miller, 1985)
Penicillin G or V (*Penicillium* sp.)	Filtration (rotary vacuum drum), solvent extraction (amyl acetate, butyl acetate, cyclic ketones)	Precipitation (potassium or sodium added), centrifugation or filtration	Drying (pre-dried with anhydrous isopropyl alcohol, butyl alcohol. Dried with warm air, vacuum or radiant heat)	(Swartz, 1985)
Polysaccharides (*Xanthamonas campestris*, *Pseudomonas aeruginosa*)	Centrifugation, milling	Precipitation (alcohol, salt and acid)	Forced air or vacuum drying	(Margaritis and Pace, 1985)
Streptomycin	Broth filtration	Precipitation, adsorption (activated carbon, non-ionic resins, alcohol, acid)		(Florent, 1985)
Thienamycin (*Streptomyces* sp.)	Pressure rotary filtration	Adsorption (Dowex 1 × 2 (HCO_3^-) resin)		(Buckland et al., 1985)
Yeasts (Bakers') (*Saccharomyces cerevisiae*, *S. uvarum*, *S. carlsbergensis*)			Freeze-, Roto-Louvre-, through circulation-, air-lift- and spray drying	(Chen and Chiger, 1985)
Yeast (*Saccharomyces cerevisiae*)	Filtration (plate and frame), centrifugation			(Smith, 1985)

[a] Separation is defined as solid liquid separation.

[b] Concentration and purification are meant as the same thing here. This is the increase in product purity by any means of downstream processing. Strictly speaking, this may also include the unit operations defined in separation and formulation.

[c] Formulation is defined as the final stage in downstream processing. It includes processes aimed at reducing the moisture content of the product. Typical examples include oven drying, freeze drying and spray drying.

Table 2 – Approximate product recoveries and concentrations in downstream processing units.

Product	Separation	Concentration	Formulation	Overall Recovery	Purity	Reference
Cephalosporins				70%	70–80%	(Smith, 1985)
Cephamycin				94%[a]		(Omstead et al., 1985)
Glycerol				50–90%	low	(Wang et al., 2001)
Gluconic acid					50%	(Milsom and Meers, 1985b)
Lactic acid		*Evaporation*: 8% (solids concentrated to 52–82%)				(Vickroy, 1985)
Lincomycin				50–90%		(Gonzales and Miller, 1985)
Penicillin G or V	*Filtration*: 90–95% *Solvent extraction*: 80–90% (single stage) 92–96% (lead trail)		*Crystallisation*: 95% *Drying*: 95%	78%		(Swartz, 1985)
Streptomycin				75-80%	99%	(Florent, 1985)
Thienamycin	*Filtration*: 80%	*Adsorption*: 66% *Other concentration*: 9–87%		36%	30%	(Buckland et al., 1985)
Yeast (Bakers')	*Dewatering*: 18–22% concentrated to 28–33% solids				*Wet basis*: 27–30% *Dry basis*: 92–96%	(Chen and Chiger, 1985)
Yeast	*Filtration*: 90% solids removed					(McCabe et al., 2005)

[a] Only includes steps AFTER filtration.

Excel model has been developed, termed the CeBER Bioprocess Modeller (Centre for Bioprocess Engineering Research at the University of Cape Town, Department of Chemical Engineering). This model allows for the use of a limited set of inputs to calculate the material and energy balance data. The approach to the generic flowsheet model is based on first principles in many instances, supplemented by data from advanced modelling studies, as well as industrial practice.

A corresponding paper described the development of the microbial growth and product formation (Harding and Harrison, 2016), while the current paper focuses on the downstream processing. Additional information can also be obtained for specific unit systems (Harding, 2009). The model allows for solid liquid separation, cell disruption, further solid liquid separation, up to six recovery, concentration and purification steps and a final formulation stage. All of these units are based on commonly used process units as shown in Table 1, with each unit having a product recovery based in part on the information in Table 2. Recovery, concentration and purification steps allow for the addition of reactive and/or non-reactive chemicals. A waste water treatment step is also included in the model. The output from the model is a material and energy balance table (Fig. 1) as well as a breakdown of the energy sources and nominal volumes of each of the units in tabular (Fig. 2) and graphical formats. This downstream processing package has application in analysing the recovery of products from microbial processes as well as conventional extraction of fine chemicals from plants and products of biocatalysis.

The model allows for fast results at an early stage of design; even by users not familiar with modelling software such as AspenPlus or SuperPro Designer. The model also includes a database and default values based on industrial norms and academic studies. Should specific information not be known, the model selects the most feasible values and automatically uses these. All automatic values can be overwritten with more suitable data as this becomes available. More detailed calculations and equations can be obtained (Harding, 2009).

2. Solid liquid separation

2.1. *Introduction*

Product recoveries and waste removals are tracked throughout the flowsheet to determine overall recoveries and purities. In each separation step, the levels of liquid and solid removal are given according to the type of separation. Depending on the phase of the product (liquid or solid), the desired flow is fed to the next unit, while the waste fraction is removed. Solid liquid separation can be achieved by centrifugation, filtration, sedimentation or a nominal 'other' method.

2.2. *Centrifugal spin and washing*

Five forms of centrifugation are provided in the model: tubular, disk, nozzle-discharge disk, helical conveyor decanter or "other", each with a specific energy requirement per volume (Perry et al., 1984). There is good agreement with this data when compared to literature values for yeasts (Bohlmann, 2002; Kalk and Langlykke, 1979; Steffens et al., 2000; Tutunjian, 1985) (Table 3). However, bacterial cultures require greater levels of energy input owing to lower settling velocities, resulting from smaller cell size.

Product Name	Penicillin V sodium crystals			**Total Product Mass**	1000 kg
				Product Mass	995.3 kg
				Purity	99.53 %
* Specific names not given				**Recovery**	96.19 %

Product Composition			**IN**			**OUT**		
Glucose	0.009394	kg	Energy/Utilities			**Total Crude Product**		
Phenoxyacetic acid	0.0009422	kg	Electricity	79.12	GJ			
Pharmamedia	0.02647	kg	Steam	3.275	ton	Product	1000	kg
Penicillin V sodium crystals	995.3	kg	Chilled water	860.4	m³			
Water	3.328	kg				**Waste**		
Sulphuric acid	0.001762	kg	Inputs			Water emissions:		
Butyl acetate	0.3823	kg	Distilled water	19.14	m³	Water	21.34	m³
Acetone	0.4651	kg	Glucose	5178	kg	Penicillium chrysogenum	903.4	kg
Sodium acetate	0.05306	kg	Phenoxyacetic acid	361.4	kg	Glucose	60.22	kg
Acetic acid	0.3566	kg	O2	29420	kg	Phenoxyacetic acid	6.04	kg
Penicillin	0.06122	kg	Pharmamedia	1301	kg	Pharmamedia	169.7	kg
			*Sulphur source	317.2	kg	Sulphuric acid	11.3	kg
			Sulphuric acid	11.3	kg	Butyl acetate	183.6	kg
			Butyl acetate	184	kg	Acetone	223.3	kg
			Acetone	223.8	kg	Sodium acetate	25.47	kg
			*Filter media 1	74.28	kg	Penicillin	128.9	kg
			Sodium acetate	259.9	kg	Acetic acid	171.2	kg
			Sodium hydroxide	113.3	kg	Penicillin V sodium crystals	39.45	kg
						Sodium hydroxide	113.3	kg
						Air emissions:		
						O2	25410	kg
						CO2	6578	kg
						SO2	151.8	kg
						Solid waste:		
						*Filter media 1	74.28	kg
						Chemical Oxygen Demand:		
						COD	3492	kg
			Total Mass	**56.58**	**ton**	**Total Mass**	**56.58**	**ton**

Fig. 1 – Example of a screenshot of the CeBER Bioprocess Modeller material and energy output.

Unit operation		Batch volume*			Electricity	Natural gas	Steam	Cooling water	Chilled water
Sterilisation	Steam sterilisation	23.26			1229		3104		
Microbial growth/Product formation		23.26							
- Aeration					10250				110.6
- Agitation					32850				
- Fermentation cooling					32510				746.5
- Post fermentation cooling					1316				
Solid-Liquid Seperation 1	(None)								
Cell Disruption	(None)								
Solid-Liquid Seperation 2	(None)								
Concentration/Purification 1	Filtration	23.27			698.1				
Concentration/Purification 2	Decanting	20.35							
Concentration/Purification 3	Centrifugation	2.5			12.75				
Concentration/Purification 4	Precipitation/Crystallisation	2.984			214		171.1		3.204
Concentration/Purification 5	Centrifugation	2.974			31.82				
Concentration/Purification 6	(None)								
Formulation	Other	0.7599			2.403				
Final product volume out		0.7106							
TOTAL					**79110**	**0**	**3275**	**0**	**860.3**
		m3	**m3**	**kg/h**	**MJ**	**m3**	**kg**	**m3**	**m3**

*Estimated working volume for each unit for a batch process i.e. total volume of material passing through the unit in order to give desired product mass

Fig. 2 – Example of a screenshot of the CeBER Bioprocess Modeller energy and nominal volume output.

Table 3 – Typical centrifuge power requirements.

Centrifuge power requirements (kJ/m^3)	Comment	Reference
Bacterial harvesting		
26 640–90 000	Axial solid ejecting centrifuge 37 kW, 1.5–5 m^3/h	(Bohlmann, 2002)
22 320	Continuous disc-type centrifuge, 37 HP motor, water removal rate 5 m^3/h	(Tutunjian, 1985)
Yeast harvesting		
2520–9000	Nozzle centrifuge 149 kW 50–200 m^3/h concentrate 10–50 m^3/h	(Bohlmann, 2002)
8280	Continuous desludging disk centrifuge, 4 m^3/h	(Kalk and Langlykke, 1979)
5400	–	(Steffens et al., 2000)
5040	Continuous disc-type centrifuge, 37 HP motor, water removal rate 5 m^3/h	(Tutunjian, 1985)

Adapted from literature (Patel, 2006).

Unless stated otherwise, the generic flowsheet model uses energy per unit volume data, calculated mass balance data and energy efficiencies to obtain energy and volume requirements. Repeat municipal, distilled or de-ionized water washing was allowed with successive washes taking into account the reduction in initial concentrations.

2.3. *Filtration*

The material and energy balance calculations for filtration assume constant pressure membrane filtration, with no cake build-up. The mass, density and flux of the material entering the filter, as well as the cross sectional area and volume of the filtration unit, are used to calculate the linear velocity of material flowing through the filtration unit. From this, together with efficiency values, the power and energy requirements are calculated. A filter media and flocculent can be added to aid in filtration efficiencies. It is assumed that the energy requirements for filtration are provided by electricity.

The model also takes into account the amount of filter material needed; based on the cross sectional area of the filter, height of filter material, density of the filter material and how often it needs to be changed. Filter media can be added as diatomaceous earth, filter paper, expanded perlite, sintered glass, wire mesh or a user specified material.

2.4. *Sedimentation*

It is assumed that there is no energy requirement for sedimentation. As with all solid liquid separation steps in the model, solid–liquid separation is based on default product/waste separation efficiency values, assumed from similar industrial units or a user defined value. A flocculent can also be added for a sedimentation unit operation – based on percentages of the total calculated volume in the unit.

3. Cell disruption

3.1. *Introduction*

Cell disruption, to release intracellular products, can be performed chemically (using *e.g.* chloroform, toluene, EDTA or lysozyme), by freeze-thaw cycling or by mechanical stress (*e.g.* cavitation, bead milling or high pressure homogenization) (Engler, 1985; Harrison et al., 1991a, b; Willson, 2010). The model allows for the most common industrially preferential mechanical disruption methods, *i.e.* high pressure homogenization (HPH), cavitation (hydrodynamic or acoustic) or using a bead mill.

Typical disruption efficiencies (%) and energy productivities (mg/J) are assumed and used to estimate appropriate default values for the model. It is assumed that product not released on cell disruption is lost on biomass separation in further processing.

3.2. *High pressure homogeniser*

The extent of cell disruption and energy efficiency (mg product released per/unit energy) on high pressure homogenization are given for *Saccharomyces cerevisiae* and *Candida utilis* (Engler, 1985) (Table S1). A basic estimation of average values is used for other micro-organisms. It is recognized that cell disruption of bacteria requires less energy than yeasts (Harrison et al., 1991a, b). It is also possible to reduce the energy requirement of disruption by chemical pretreatment of the suspension (Anand et al., 2007; Bailey et al., 1995).

3.3. *Cavitation*

Cavitation data are split into hydrodynamic and acoustic cavitation. Energy efficiency values by mass or volume (mg/J or J/ml) obtained literature (Save et al., 1994; Senthil Kumar et al., 2000) have been used in the model. Additional literature on cavitation for the total or partial release of intracellular material have been reported (Balasundaram and Pandit, 2001; Balasundaram and Harrison, 2006; Gogate and Pandit, 1985; Save et al., 1997; Sundaram et al., 2003).

3.4. *Bead mill*

Cell disruption (%) and energy efficiencies (mg/J) used in the model are given for a bead mill. Data are given for *S. cerevisiae*, *Saccharomyces carlsbergensis* and *C. utilis* (Engler, 1985) (Table S2). Additional literature on mills have been reported (Agerkvist and Enfors, 1990; Melendres et al., 1993; Woodrow and Quirk, 1982), but these do not include the required data for the model.

4. Concentration and purification

4.1. *Introduction*

A maximum of six concentration and purification units can be modelled to achieve a desired product purity from solution. These include adsorption, centrifugation, chromatography, decanting, evaporation, filtration, precipitation/crystallization, solvent extraction and splitters.

Material balancing for centrifugation and filtration is based on solid and liquid fractional removals. For precipitation/crystallization, it is assumed that no product is lost or waste removed since all material flows to the next unit. For adsorption, chromatography, solvent extraction and decanting, it is assumed that 99% of the product phase is retained and 95% of the waste phase is removed. All default numbers are estimations based on ideal conditions; however, can be changed. It is further assumed that all phase changes are complete.

In the separation steps, additional materials may be added to facilitate separation through, for example, phase changes, ionic modification, flocculation *etc.* It is assumed that these materials do not react, but flow through the system in the same manner as other materials. Further, two reactants can also be added to facilitate separation. Following reaction, the products formed also separate in the same manner as other materials. The reacting and non-reacting chemicals accommodated by the model include over 40 common substances and also allows for the user to specify other chemicals.

4.2. *Adsorption and chromatography*

The required energy for both adsorption and chromatography is calculated from first principles based on the pumping requirement to contact the liquid across a cross sectional area, under a given pressure. Default length to diameter ratios, pumping efficiencies and pressure changes are all assumed (and can be changed) in calculating the volumes needed to determine the cross sectional area.

4.3. *Centrifugation*

The material and energy balance for centrifugation uses the approach presented above under solid–liquid separation. Since the need for centrifugation during concentration or purification is more limited than in solid–liquid separation, the current model approach is simplified. No choice of type of centrifuge is allowed. Instead, the average power per unit volume across various types of centrifuges is used as default. Repeat centrifugation and wash cycles are not accommodated, but should more than one centrifuge step be needed, using an additional centrifugation in the next unit is recommended. Further developments to the model will include the same approach as in solid–liquid separation.

4.4. *Evaporation*

The energy required to heat and evaporate water from the product is calculated from an energy balance calculation, using the specific heat capacity, the heat of vaporization, temperatures and efficiencies. These values should be similar to those shown in Table S3. Potential for energy integration within the system, as well as multi-effect evaporation, is currently not included. These would both reduce energy requirements.

It is assumed that the energy required for heating is obtained from natural gas. If natural evaporation is required, the unit operation 'other' should be chosen and the energy per unit volume value set to zero.

4.5. *Filtration*

Filtration operations for concentration and purification used in the generic flowsheet model are more sophisticated than those required for solid–liquid separation. Five subcategories are considered. Each has an associated energy per unit volume requirement (diafiltration: 18-, microfiltration: 7.2-, nanofiltration: 252-, reverse osmosis: 32.4-, and ultrafiltration: 20.2 MJ/m^3 (Patel, 2006)), used to calculate the energy requirement. This energy requirement should correspond to a pressure drop lower than the suggested limit of 3 atm (constant pressure filters) as recommended in literature (McCabe et al., 2005). Material balance calculations are performed as in filtration calculations of the solid–liquid separation units.

The diafiltration option allows for the addition of a diafiltration solution (salt and water). As with filtration for solid–liquid separation, there is allowance for a flocculent and provision for the filter media used.

4.6. *Precipitation or crystallization*

Electrical energy requirements for precipitation or crystallization are based on the energy requirement for agitation, with a default power per unit volume value of 0.8 kW/m^3 (a mild to medium mixing value for precipitation (Coulson and Sinnott, 1999)) and an electrical to mechanical efficiency of 80% assumed. The unit can be heated with steam and cooled again before further processing. Both the steam and cooling water needed for this are included in material balance calculations. The calculation also takes into account temperatures, temperature differences, residence times, specific heats and densities.

In modelling the precipitation or crystallization unit, precipitating chemicals are assumed to undergo a perfect phase change from liquid to solid. The solids formed can then be removed in further processing (*e.g.* filtration or centrifugation) as desired to further purify the product.

4.7. *Solvent extraction/decanting*

Modelling of solvent extraction requires the addition of a solvent and the defining of two phases: product and waste. The product phase moves on to the next unit operation, while the chemicals in the waste phase and the remaining liquid phase are removed according to the separation efficiency specified. A similar approach is taken for decanting. However, no solvent is added, but two phases and the same separation method are used.

4.8. *Splitter*

An arbitrary unit to simply divide a stream into product (for further purification) and waste is also included. The splitter is simply defined by a split ratio of product to waste, with no energy requirements included. The splitter is an addition in the model to allow for purging a stream or to manually account for losses where energy inputs are minimal or unknown.

5. Formulation

5.1. *Introduction*

The final step in the generic flow sheet model is formulation. This can take the form of oven-, spray- or freeze-drying. Typical examples and conditions are shown in Table 4. Energy is supplied as natural gas for spray drying and oven drying or electricity for freeze-drying. It is assumed that 99% of the product is retained in the formulation step and that 99%

Table 4 – Typical literature values for different drying methods.

Drying method	Steam and electricity requirements		Temperature	Time	Drying rate (Final moisture content)	Comments	Reference
	kg steam/kg evaporated	kWh/kg	°C	min	kg water/h (%)		
Belt dryer	1.38	–				Approx. 60 °C	(IPTS, 2003)
Co-current drum dryer	1.76	–				Evap. of 46 t/h water	(IPTS, 2003)
Fluidised bed dryer	0.24	–				25 bar steam produces 3 bar heating steam	(IPTS, 2003)
Spray dryer	3	0.1				–	(Bartholomew and Reisman, 1979)
Spray dryer	1.62–2.33	–				1, 2 & multistage dryers	(Patel, 2006)
Spray dryer	2	–					(Gerngross, 1999)
Tower dryer	2.0–2.4	0.1				No heat recovery	(Reisman, 1988)
(Unknown)	1.2–1.67	0.25–1				Drying of food	(IPTS, 2003)
Air-lift dryer			100–150	10–240	160–350 (7)		(Chen and Chiger, 1985)
Freeze drying			−35				(Chen and Chiger, 1985)
Heated air			38		(7–8)		(Chen and Chiger, 1985)
Roto-Louvre drying			50–60	600–1200	5400	Size: 4.85 × 2.2	(Chen and Chiger, 1985)
Spray dryer			In: 100–120 Out: 65–67			(5–6)	(Chen and Chiger, 1985)
Through circulation dryer			28–50				(Chen and Chiger, 1985)

of the moisture is removed in this unit. Typical final moisture contents can be as low as 2% in baker's yeasts, with dryer temperatures as high as 150 °C (Chen and Chiger, 1985). The heat lability of many bioproducts implies that careful control of the effective temperature of the product, and time of exposure, is required.

5.2. *Oven drying*

The energy required to heat material and evaporate moisture by oven drying is calculated from first principle energy balance calculation, using natural gas as the energy source. Energy calculations are similar to those for evaporation used elsewhere in the model.

5.3. *Freeze drying*

The energy to freeze dry the product is determined by the energy needed to cool the liquid to freezing, to freeze and subcool it, and the energy to create a vacuum in the container. It is assumed that no external energy inputs are required to sublimate the liquid from the product. The calculation includes all temperatures, the freeze drier inlet area, efficiencies, specific heats of water and ice and the latent heat of freezing. The energy required for vacuum pumping is calculated as for other pumping requirements in the model (see adsorption and chromatography).

5.4. *Spray drying*

Energy data for spray drying is calculated according to an energy balance given in literature for spray drier energy requirements (Baker and McKenzie, 2005). In the model, a simplification is made that the thermal loss factor is zero (i.e. adiabatic system) (Baker and McKenzie, 2005; Keey, 1992). This calculation requires inlet and heated temperatures, humidities, specific heat of air, latent heat of vaporization, as well as the density and lower heating value of natural gas, the assumed energy source for spray drying.

6. Wastewater treatment

A simplified wastewater treatment scenario is included whereby wastewater collected in the discarded streams may be neutralized by the addition of up to three chemicals. A stoichiometric oxygen demand (StOD) value (IChemE, 2003), has been included and can be used as a water pollution value. Opportunities exist to expand the model to include more appropriate wastewater treatment scenarios; including anaerobic digestion and other systems.

7. Case study: penicillin V production

7.1. *Downstream processing*

After penicillin production (Harding and Harrison, 2016), the fungal culture was transported to a rotary vacuum filter where the fungal biomass was removed and washed with water (Fig. 3). Sulphuric acid was added to reduce the pH to approximately pH 3 and the temperature was lowered to minimize penicillin degradation. The penicillin was transferred to an organic phase following addition of butyl acetate. Thereafter centrifugation allowed the removal of the aqueous

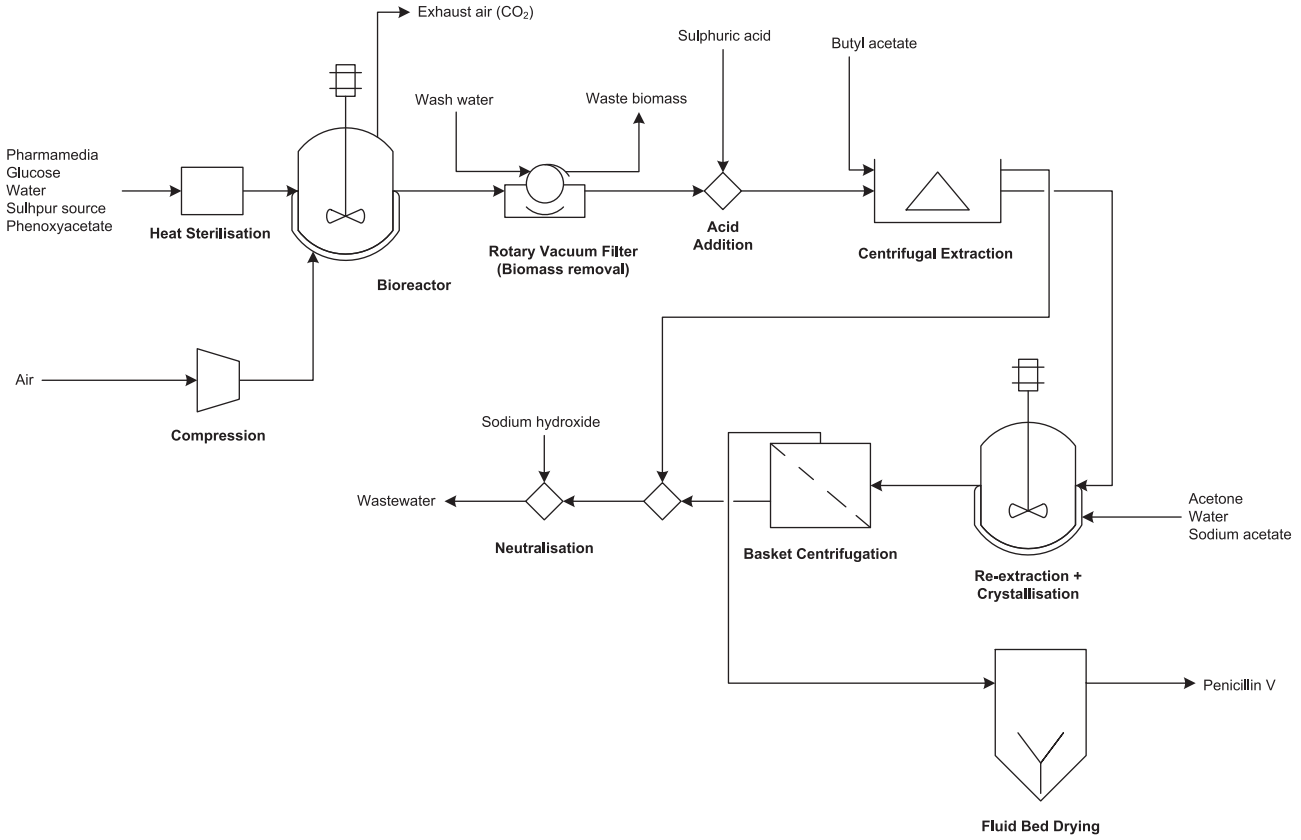

Fig. 3 – Simplified process flowsheet for penicillin V sodium salt production as modelled in the MS-Excel model (simplified from literature (Biwer et al., 2005; Heinzle et al., 2006)).

solution. Not shown, butyl acetate may be recycled from this stage in more complex designs.

Sodium acetate, acetone and water were added to the penicillin V solution for its re-extraction into the acetone/water phase and subsequent precipitation as crystals of the sodium salt. These were washed, separated in a basket centrifuge and air-dried in a fluidized bed dryer. The wash solution from the basket centrifuge as well as the previous centrifugal extraction wastewater was neutralized with sodium hydroxide before being discharged to effluent treatment. In a more optimum system, it may be possible to recycle sodium acetate or acetone and allow for water re-use.

7.2. *Process simulation results*

The material and energy balance results were compared to a Penicillin V model presented in literature (Biwer et al., 2005; Heinzle et al., 2006). The amount of glucose needed was less than 1% higher in the CeBER Bioprocess Modeller results (Fig. 4). The amount of Pharmamedia used in the generic

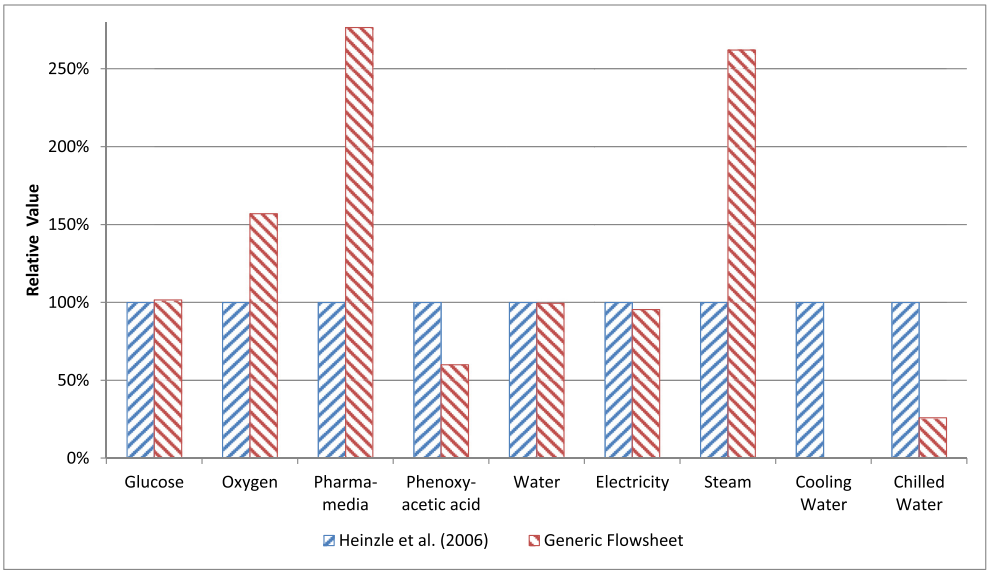

Fig. 4 – Relative comparison of selected material, energy and utility inputs for Penicillin V sodium salt production from literature (Heinzle et al., 2006) versus the generic flowsheet model.

Table 5 – Material flows for the production of Penicillin V (literature) *vs.* results from the generic flowsheet model developed.

Component	In (kg)	Out (kg)	In (kg)	Out (kg)
	Heinzle et al. (2006)		Generic flowsheet	
Acetic acid	–	0.17	–	0.17
Acetone	0.12	0.12	0.22	0.22
Biomass (dry cell weight)	–	0.88	–	0.90
Butyl acetate	0.32	0.32	0.18	0.18
Carbon dioxide	–	5.47	–	6.58
Glucose	5.10	0.10	5.18	0.06
Oxygen (excl. excess & N_2)	2.56	–	4.02	–
Penicillin V (loss)	–	0.10	–	0.13
Penicillin V sodium salt	–	1.00	–	1.00
Penicillin V sodium salt (loss)	–	–	–	0.04
Pharmamedia	0.47	0.06	1.30	0.17
Phenoxyacetic acid	0.60	0.01	0.36	0.01
Sodium acetate	0.23	0.01	0.26	0.03
Sodium hydroxide	0.12	0.12	0.11	0.11
Sulphur source	–	–	0.32	–
Sulphuric acid (DSP additive)	0.01	0.01	0.01	0.01
Trace metals	0.77	0.10		
Water	19.2	21.1	19.1	21.3
Product yield (kg pen/kg glucose input)		0.20		0.19
Product recovery (% kg pen cr.)		99.0		96.2

flowsheet scenarios was almost 3-fold higher, while phenoxyacetic acid was 40% lower than for literature (Biwer et al., 2005; Heinzle et al., 2006), (Table 5). However, these values differed as the literature analysis assumed industrial norms, while in the model presented, the required amount was calculated from stoichiometric ratios. When these values are taken as a combined input, the model is only 55% higher than the industrial norms. The oxygen requirement was 57% higher in the model due to conservative overestimations used.

For each kilogram of penicillin produced, approximately 19 kg of process water was required. This showed good agreement with the amount of water shown to be required in literature (Heinzle et al., 2006). The amount of steam predicted by the CeBER Bioprocess Modeller was higher but electricity was lower (approximately 0.9 fold) (Table 6). This was a result of steam sterilisation in the generic flowsheet models versus heat sterilisation, by electricity, in literature (Heinzle et al., 2006). The total energy equivalent was comparable (88.03 *vs.* 86.38 MJ/kg penicillin respectively) to the literature value.

Table 6 – Energy and utility flows for the production of Penicillin V (literature) *vs.* results from the generic flowsheet model developed.

Energy requirements	Heinzle et al. (2006)	Generic flowsheet	Units
Electricity	23.04 (82.9)	22.0 (79.12)	kWh/kg pen (MJ/kg pen)
Steam (140 °C, 3 bar)	1.26 (3.4)	3.3 (8.91)	kg/kg pen (MJ/kg pen)
Total energy equivalent	86.38	88.03	MJ/kg penicillin
Chilled water	3.32	0.86	m^3/kg penicillin
Cooling water	1.17	–	m^3/kg penicillin

Butyl acetate requirements were reduced to 43% and acetone requirements increased 3 fold because of simplifications on a recycle stream. Other inputs and outputs were within 10% of the literature values.

8. Conclusions

This paper provides a generic flowsheet model (CeBER Bioprocess Modeller) for fast, first estimate material and energy balance inventories of industrial bioprocesses. Presented in an MS-Excel format, the model uses a stoichiometric approach, together with first principles and rules of thumb. The CeBER Bioprocess Modeller allows for batch or continuous production by aerobic or anaerobic and intra- or extracellular means, the details of which are presented in an accompanying paper (Harding and Harrison, 2016). Downstream processing units were included to allow for accurate representation of typical process setups for biomass separation, cell disruption (where needed), product recovery, concentration, purification and formulation. In this paper, following review, appropriate approaches to the modelling of each unit operation are described, with indication of simplifying assumptions and opportunity to expand the level of detail. Typical data required for these analyses have been collected from bioprocess systems and are stored in a database. These include relevant constants and physical data that are required by the model. The framework of the CeBER Bioprocess Modeller ensures that maximum flexibility is maintained in terms of process flowsheets that can be analysed using this tool. From the outputs in the model, techno-economic evaluations or environmental analyses, *e.g.* life-cycle assessments, could be performed, since all required information (mass/energy balance data, vessel sizes and more) is calculated and provided.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.sajce.2016.10.002.

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