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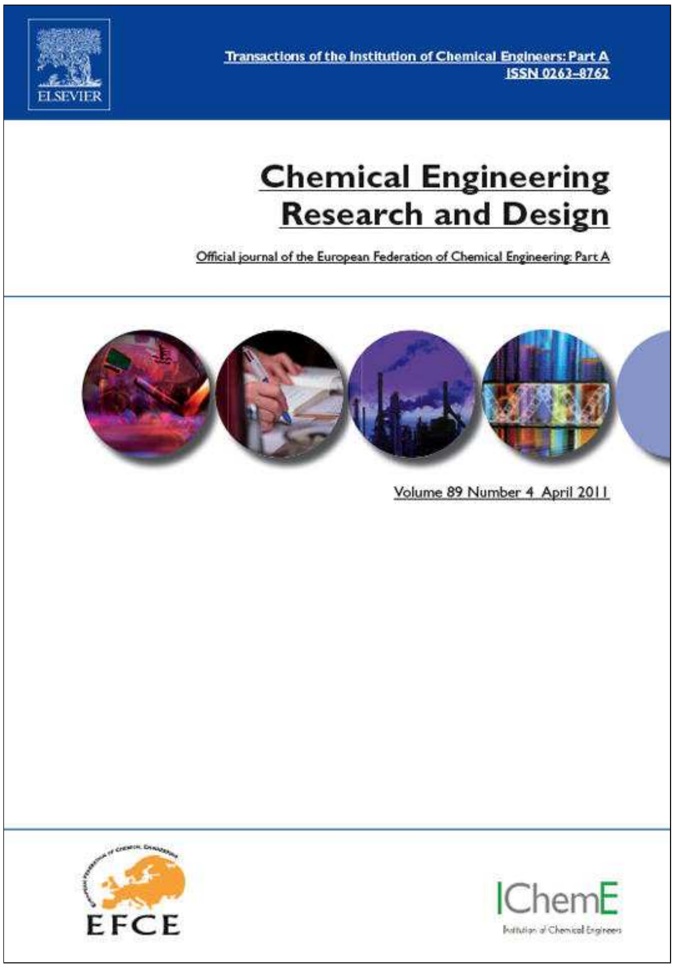
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Simulation, scale-up and economics of adsorption and membrane based processes for isoflavones recovery☆

*Nikhil Kawachale, Ashwani Kumar**

Institute for Chemical Process and Environmental Technology, National Research Council of Canada, 1200 Montreal Road, Ottawa, Ontario, K1A 0R6, Canada

ABSTRACT

Laboratory scale adsorption and membrane based processes for the recovery of isoflavones from red clover flowers were reported earlier (Xu et al., 2005, 2006). However, studies on scale-up and simulation for commercialization for both the methods have not been studied. In present study the economic feasibility of isoflavones recovery based on these two methods was investigated with a commercial bio-process simulator (SuperPro Designer®). Laboratory scale separation data from previously published work was used in the simulations. Preliminary simulation studies confirmed the requirement of effluent streams recycling in order to reduce the production costs. Different flowsheets incorporating solvent recovery and recycle operations were designed and simulated to compare the economics of operation. Modified adsorption and membrane based processes incorporating recycling of waste streams were found to be economically more attractive than that of respective standard processes reported in the literature. The membrane process with solvent recycling had the lowest production cost of US$618/kg for isoflavones supplement. The adsorption-recycle process was found to be more expensive at US$1116/kg, while it had a higher content of isoflavones.

Keywords: Isoflavones; Red clover flowers; Adsorption process; Membrane process; Process simulation

1. Introduction

Isoflavones are secondary plant metabolites (polyphenols) and belong to a group of natural products called flavonoids. Nutraceutical products such as isoflavones from plant sources elicit a large number of physiological responses in humans and other mammals (Vacek et al., 2008). Isoflavones are well accepted as anti-carcinogenic substances and they have found several applications in the prevention of osteoporosis and cardiovascular diseases (Setchell, 1998; Ososki and Kennelly, 2003). Although soybeans are generally the major source of isoflavones, lately red clover plant has received increased attention for significant isoflavones content in its flowers. The total isoflavone content including genistein, genistin, biochanin-A and formononetin in red clover flowers is 2590 μg/g, which is higher than those reported for soybeans and soy products (Chang, 2002). While nutritional studies indicate that the adsorption in humans of isoflavones from red clover flowers and soybeans is comparable (Tsunoda et al., 2002), red clover-derived isoflavones do not show any estrogenic activity thus making them superior to soy-based isoflavones in hormone replacement therapy (Atkinson et al., 2004). Moreover, the possible application of isoflavones from red clover flowers as an antioxidant in functional foods is supported by relatively higher capacity of red clover extract than that of a soy preparation (Kroyer, 2004).

Consequently there is a potential demand for isoflavones recovered from red clover flowers as concentrates and isolates. Isoflavones recovered from red clover flowers by utilizing the same methods developed for soybeans often require large quantity of organic solvents in conjunction with various chromatographic techniques, which entails longer processing time and are not cost effective (Chang, 2002). More efficient and economical processes based on adsorption and membrane-separation were discussed in earlier studies, where the product could be directly used as isoflavone supplements or

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* *Corresponding author*. Tel.: +1 613 998048.
E-mail address: ashwani.kumar@nrc-cnrc.gc.ca (A. Kumar).

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further purified to obtain isoflavone isolates (Xu et al., 2005, 2006). An adsorption based approach reported by Xu et al. (2005) comprises four major steps: extraction, adsorption, elution and drying in order to recover isoflavones. The technique was developed to produce a final product with isoflavones content of approximately 20% that was accounted for more than 50% of the total isoflavones in red clover flowers. A more straight forward membrane based approach was reported for isoflavones processing from the red clover flowers (Xu et al., 2006). The resulting product in membrane based process had 9% isoflavones, which represented about 35% of the total isoflavones content in red clover flowers and may be used as an isoflavones supplement without further processing.

However, both the adsorption and membrane based processes were studied at laboratory scale and no information is available on expansion and commercialization. Studies on comparison of different alternatives which might lead to an identification of a more suitable process are lacking as well. The objective of present study was to design processes and evaluate the economics of both adsorption and membrane based processes to recover isoflavones from red clover flowers through intergraded material/energy balances, equipment sizing, utility requirements and waste stream recycling.

2. Materials and methods

The computer-aided process design and simulation tools can facilitate development, evaluation, scale-up to perform cost analysis in order to determine indicative production costs of isoflavones under realistic scenarios. Moreover, process simulators offer an opportunity to shorten the time required for process development and allow comparison of process alternatives so that number of process alternatives can be implemented and analyzed in a short duration (Rouf et al., 2001). In contrast to petrochemical and other industries that handle large throughputs and utilize continuous processes, most bio-processes operate in a batch or semi-continuous mode (Papavasileiou et al., 2007). SuperPro Designer® from Intelligen, Inc. (Scotch Plains, NJ) in this respect is a recipe driven tool that account for time dependency and sequencing of events. The system facilitates the user to concurrently design and evaluate manufacturing with end-of-pipe treatment processes and waste minimization. The potential application of commercial batch process scheduling and simulation tool throughout the life-cycle of development and commercialization of pharmaceutical products such as tablets was demonstrated by Papavasileiou et al. (2007). Misailidis et al. (2009) successfully utilized SuperPro Designer® in order to examine three bio-processing alternatives designed for co-production of arabinoxylan in a wheat biorefinery operation. It was concluded that the economics of the biorefinery improved as a result of extraction of arabinoxylan as a co-product and incorporation of recycling loop for other critical components. Kotoupas et al. (2006) investigated the economic evaluation and environmental impact assessment for the treatment of cheese whey wastewater through computer-aided process design. In this work it was shown that different process scenarios including membrane based unit procedures such as ultrafiltration and reverse osmosis could be successfully modeled using SuperPro Designer®.

The modeling of an integrated process with SuperPro Designer® is initiated with flowsheet development, which represents an overall process. The flowsheet development is incorporated by collectively placing the required unit procedures and connecting them with material flow streams. Subsequently, the flowsheet has to be initialized by registering various materials that are being used in the process and specifying operating conditions and performance parameters for various operations. In SuperPro Designer®, the set of operations that comprise a processing step is referred as a unit procedure as opposed to unit operation and is represented with an icon similar to equipment. The individual tasks contained in a procedure (charge, heat, agitate, and others) are referred as operations. Essentially, a unit procedure is the recipe that describes the sequence of actions required to complete a single processing step. For every operation within a unit procedure the simulator includes a mathematical model, which performs material and energy balance calculations and based on the material balances equipment-sizing calculations are performed. In case of multiple operations within a unit procedure would dictate different sizes for a certain piece of equipment; the software reconciles different demands and selects an equipment size that is appropriate for all operations with minimum capital costs. If the equipment size is specified by the user, the simulator verifies that the vessel is not overfilled during any operation. The tool confirms that the vessel contents will not drop below a user-specified minimum volume for applicable operations as well. The capital costs as well as operating cost were estimated as a part of project economic evaluation. The equipment cost is estimated based on built-in power law model and the fixed capital investment is derived from the equipment cost and equipment/process specific multipliers (*e.g.* installation, piping) (Rouf et al., 2001; Papavasileiou et al., 2007). The annual operating cost is calculated by appropriate cost factors for utility requirement and the number of labor-hours.

3. Simulations and results

Adsorption and membrane based processes were assessed with SuperPro Designer® for evaluation of large-scale production of isoflavone concentrates. In case of both methods, the flowsheets were designed and simulated to process different amounts of dry red clover flowers. The equipment occupancy together with recipe scheduling was regulated for a standard eight hours batch. The supplementary input streams were adjusted accordingly so that the concentration and distribution of individual components across the streams matches and the model verifies with the reported laboratory scale process (Xu et al., 2005, 2006). In all cases, the entire simulation was set in a design mode, where the program determined the size of equipment based on input streams and would select more than one piece of equipment based on the physical limitations.

3.1. *Adsorption process*

The development of adsorption process was based on the data available in the literature (Xu et al., 2005) and was modeled as a batch recipe as shown in Fig. 1. Each production run starts with dried red clover flowers mixed with water at a solvent-to-flower ratio of 40:1 and at atmospheric pressure. The alkaline extraction of isoflavones at elevated temperatures (90 °C) and at pH 10.0 by adding 4 M NaOH was carried out in a reactor vessel procedure (R-101). As discussed earlier the reactor vessel unit procedure was incorporated with heat, agitate and charge unit operations. The extract was then strained through plate

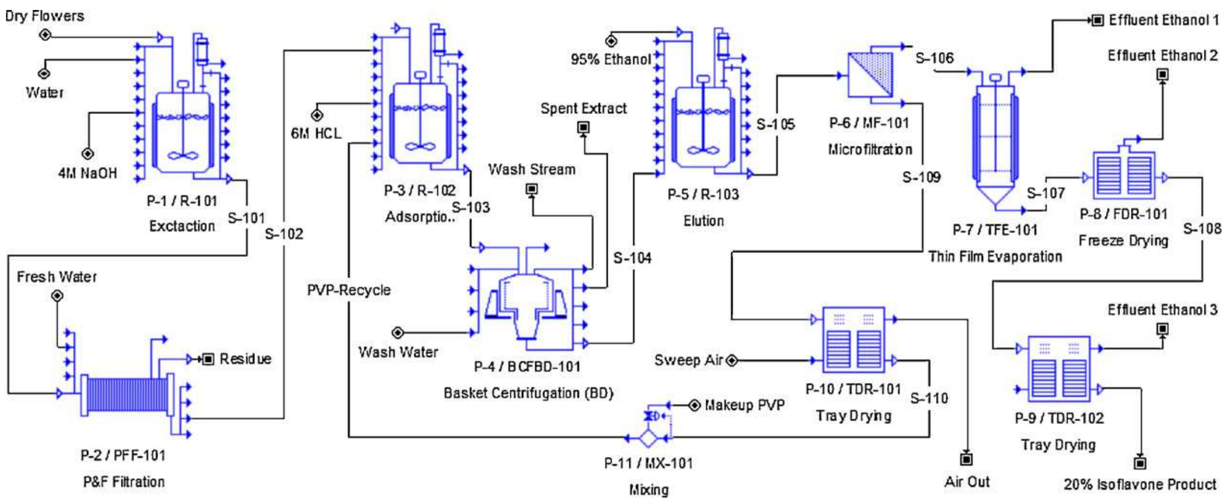

Fig. 1 – Process flowsheet for scale-up adsorption based isoflavone recovery.

and frame filter (PPF-101) at ambient conditions to separate solid residue. In an adsorption unit procedure (R-102), extract was then mixed with polyvinylpolypyrrolidone (PVP) adsorbent at an adsorbent-to-flower ratio of 0.15. The adsorption was carried out at an ambient temperature/pressure and at pH 5.5 by adding 6 M hydrochloric acid (HCL). The adsorption procedure was modeled by integrating cool, agitate and charge unit operations in a reactor vessel (R-102) unit procedure. The slurry was then passed to basket centrifuge procedure (BCFBD-101) in order to separate PVP, which was included with filter and cake-wash unit operations to separate and wash the PVP with water (equivalent to 15% of original extract) before an elution step. The isoflavones adsorbed on PVP were eluted in a reactor vessel procedure (R-103) at standard conditions with 95% (v/v) ethanol solution, which was equivalent to 15% of original extract. The ethanol solution from elution step was then filtered in microfiltration unit procedure (MF-101) with concentrate unit operation in order to separate solid PVP. The separated PVP from microfiltration unit was then air dried at room temperature in a tray-drying unit procedure (TDR-101) through dry unit operation, which was set for 100% removal of volatile components. The PVP from try-drying unit procedure was then recycled to reaction vessel unit procedure (R-102) for the next run. The custom mixing unit procedure (MX-101) was integrated in PVP recycle stream, which automatically compensated for the fraction of PVP that has been lost in previous procedures. The ethanol filtrate from microfiltration unit was evaporated under vacuum and at 60 °C in a thin film evaporator (TFE-101) where the evaporation/phase splits were calculated based on Vapor Liquid Equilibrium (VLE) model. The lyophilization of aqueous portion from evaporator was accomplished at 0 °C with freeze drying unit procedure (FDR-101), where the volatile component evaporation in dry unit operation was calculated based on Loss on Drying (LOD) model. The residue from the freeze drying was dried at 105 °C in a tray-drying unit procedure (TDR-102) to obtain the isoflavone rich product. Also, plate and frame filtration, basket centrifuge and microfiltration unit procedures were included with Clean in Place (CIP) unit operation following the main processing steps.

3.2. *Adsorption process with recycle*

A major drawback, after performing the simulation runs on standard adsorption process, was identified as effluent ethanol streams from evaporation, freeze drying and tray-drying unit procedures. In order to develop an economically attractive process, recycling of effluent ethanol vapors after condensation could be considered as a desirable option. As a result, the standard adsorption process was modified as shown in Fig. 2, where the supplementary unit procedures (Red) and the required streams (Gray) are shown along with the original unit procedures (Blue) and coupled streams (Black). The vapors from the steps discussed above (TFE-101, FDR-101, and TDR-102) contained ethanol and water except the tray-drying operation (TDR-101) for PVP, which had ethanol vapors and air as an effluent. As shown in Fig. 2, in a modified adsorption process with recycle, the vapors from all these operations were sent to a mixing unit procedure (MX-102) before they were condensed in a condensation unit procedure (HX-102). The HX-102 unit procedure was set with chilled water as a cooling agent and the condensation/phase splits was calculated using VLE model for vapor/liquid phase. The ethanol solution was further concentrated to 95% (v/v) in a distillation unit procedure (C-101) and later cooled to an ambient temperature in a cooling step (HX-102) to meet the requisite ethanol-stream specifications (Xu et al., 2005). Steam as a heating agent for re-boiler, and cooling water as a cooling agent for condenser were selected in a distillation unit procedure (HX-102) with an operating temperature of 102 °C. The cooled ethanol was then supplied to elution step (R-103) through a custom mixing unit procedure (MX-103) where make-up ethanol was repeatedly added to compensate for the cumulative losses.

3.3. *Membrane process*

The membrane based process as shown in Fig. 3 was modeled as a batch recipe using literature data (Xu et al., 2006). Each batch was mixed with an ethanol solution at solvent-to-flower ratio of 40:1 at ambient conditions in a reactor vessel unit procedure (R-101), which was integrated with charge and agitation unit operations. Ground red clover flowers were extracted with 50% (v/v) ethanol solution and the extract, after straining through plate and frame filter (PPF-101) at standard temperature/pressure, was concentrated with an ultrafiltration unit procedure (UF-101) at trans-membrane pressure of 550 kPa until the feed volume was reduced by about 90%. The permeate stream of ultrafiltration step was further concentrated with reverse osmosis unit procedure (RO-101) at a

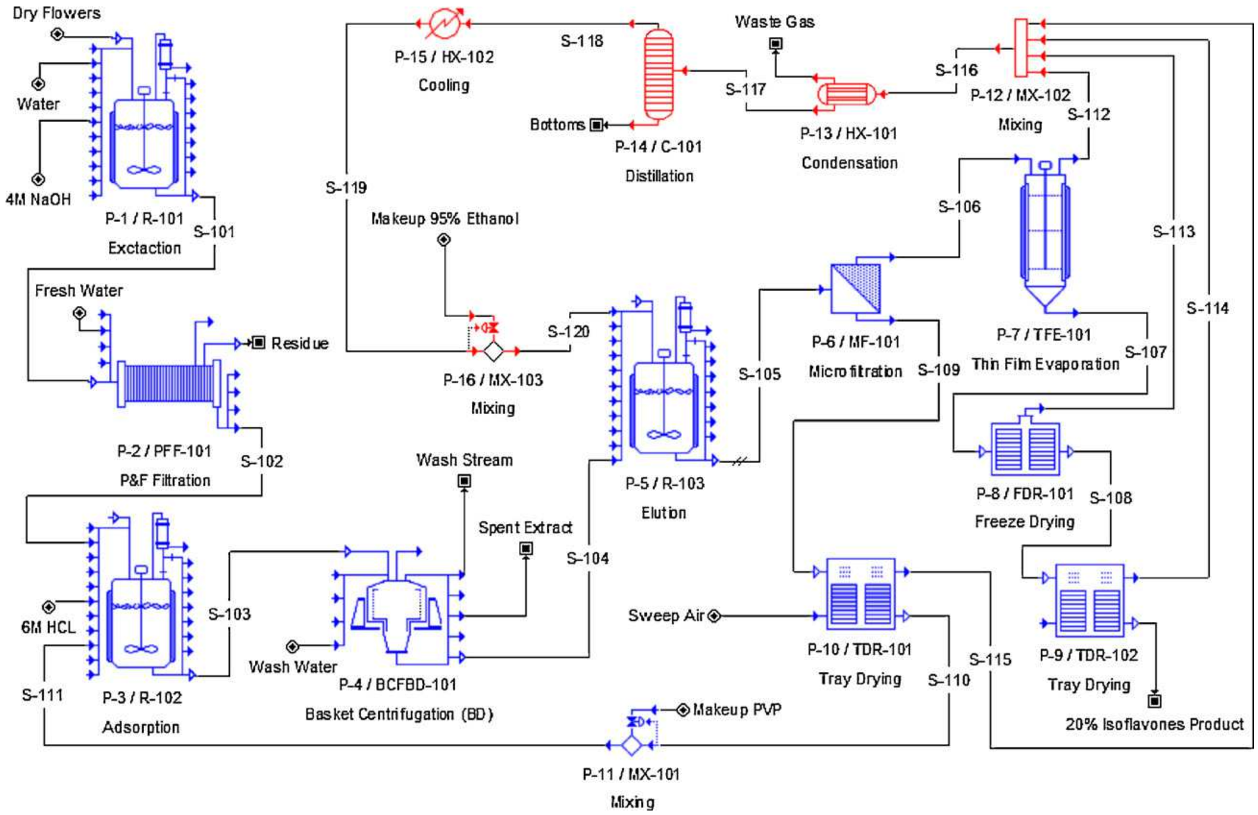

Fig. 2 – Process flowsheet for scale-up adsorption based isoflavone recovery with recycle.

trans-membrane pressure of 1800 kPa and at room temperature. The concentrate from RO unit was then evaporated at atmospheric pressure and at 90 °C in a thin film evaporator procedure (TFF-101) until the volume was reduced to about 1/3 that of the original reverse osmosis retentate. The evaporation/phase splits operation in an evaporator procedure was calculated with VLE model. The aqueous portion from an evaporator was then transferred to hold/decanting unit procedure (V-101), which was integrated with cool, hold and split unit operations. In a cool unit operation, the concentrated residue was subsequently refrigerated at 4 °C to allow micelles formation. After decanting the supernatant in split unit operation, the micelles were dried in a freeze drying unit procedure (FDR-101) to obtain the isoflavone rich product. The volatile component evaporation in dry unit operation in freeze drying unit procedure (FDR-101) was calculated based on final LOD model. As discussed in adsorption based process, CIP was integrated with plate and frame filtration, ultrafiltration and reverse osmosis unit procedures.

3.4. *Membrane process with recycle*

Similar to the adsorption based process a significant shortcoming of standard membrane process (Fig. 3) was identified as effluent ethanol vapors from evaporation (TFE-101) and freeze drying (FDR-101) unit procedures. Moreover the waste

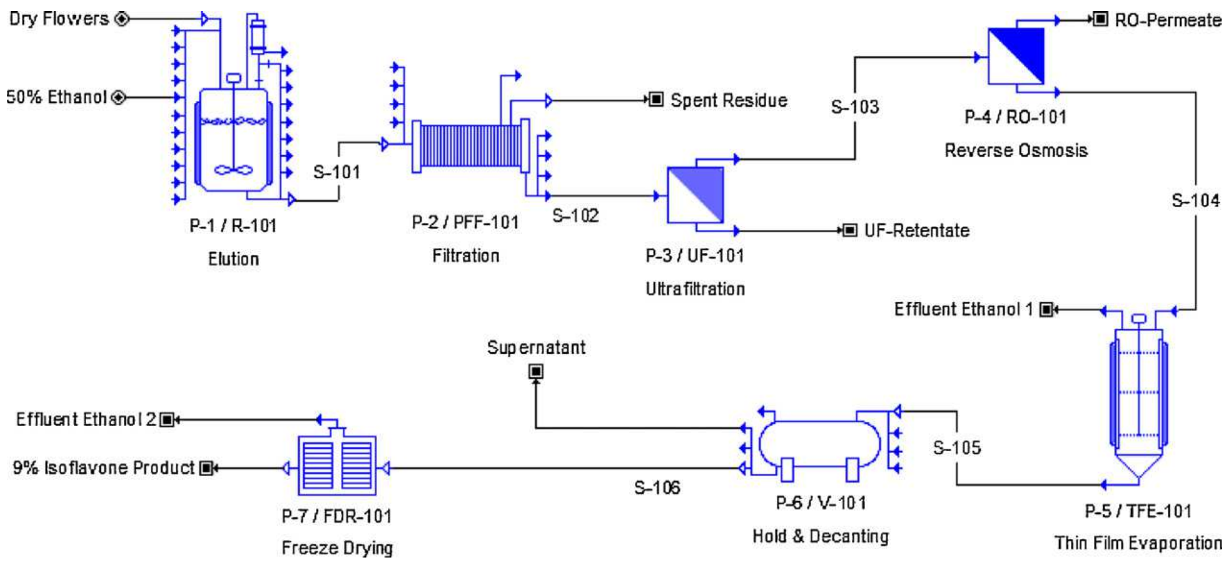

Fig. 3 – Process flowsheet for scale-up membrane based isoflavone recovery.

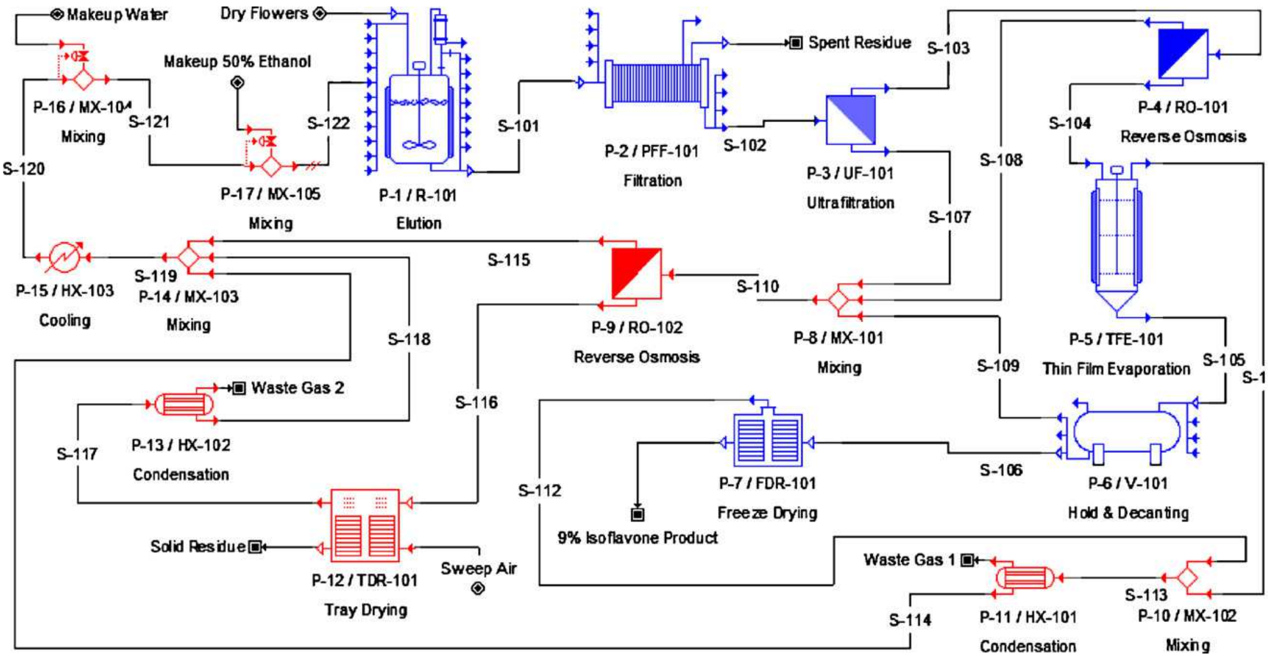

Fig. 4 – Process flowsheet for scale-up membrane based isoflavone recovery with recycle.

streams from ultrafiltration (UF-101), reverse osmosis (RO-101) and hold/decanting (V-101) unit procedures had liquid ethanol as a major component. Considering the fact that 50% (v/v) ethanol solution was consumed at a solvent-to-flower ratio of 40:1 for elution of isoflavones, recycling of ethanol from the waste streams could be economically critical for larger batch sizes where ethanol requirement would be considerably higher. Consequently, the original membrane process was modified with recycle streams as shown in Fig. 4. As discussed earlier, the supplementary unit procedures (Red) with the required streams (Gray) and the standard unit procedures (Blue) along with linked streams (Black) are represented in different colors for easy understanding. The effluents from TFE-101 and FDR-101 were directed to the mixing unit procedure (MX-102) before they were condensed in the condensation unit procedure (HX-101). The HX-101 unit procedure was set with a condensation temperature of 25 °C with chilled water as a cooling agent and the condensation/phase splits was calculated using VLE model for vapor/liquid phase. Also, the liquid waste streams from UF-101, RO-101 and V-101 were supplied to the second reverse osmosis unit (RO-102) after processing in a mixing unit procedure (MX-101) at an ambient temperature. The rejection coefficient and recovery (permeate/feed) for RO-102 procedure were set as 1 and 89.3% respectively for both isoflavones and inert flower mass, which were present as impurities in reject streams. In order to attain minimum ethanol waste, the retentate stream (S-116) from reverse osmosis unit (RO-102) was further processed in a tray-drying unit procedure (TDR-101), where the volatile components from the stream were evaporated as an effluent. The volatile component evaporation in a dry unit operation in tray-drying unit procedure (TDR-101) was calculated based on final LOD model in order to recover around 99% ethanol/water and air was utilized as a sweep gas during drying. Afterwards the effluent vapors (ethanol and water) from TDR-101 were condensed in a condensation unit procedure (HX-102), which was set with a condensation temperature of 25 °C, chilled water as a cooling agent and VLE model for vapor/liquid phase calculations. The condensed vapors from HX-102 were mixed with other recovered ethanol solution streams from RO-102 and HX-101 unit procedures in a mixing unit procedure (MX-103). The ethanol solution from MX-103 was then cooled at room temperature in a cooling step (HX-103). The ethanol solution was then recycled to an elution step (R-101) through custom mixing unit procedures (MX-104 and MX-105) in order to adjust ethanol concentration at 50% (v/v) as described in the literature (Xu et al., 2006). The custom mixing procedures MX-104 and MX-105 were individually compensated for the water and ethanol that has been lost in previous procedures respectively.

3.5. *Cost analysis and economic evaluation*

As a major capital expenditure, cost analysis and project economic assessment were important considerations in plant design. Since there was no definitive information available for the market price of isoflavone supplements and the revenues generated, the break even analysis and return on investment (ROI) calculations were not performed and the estimated unit

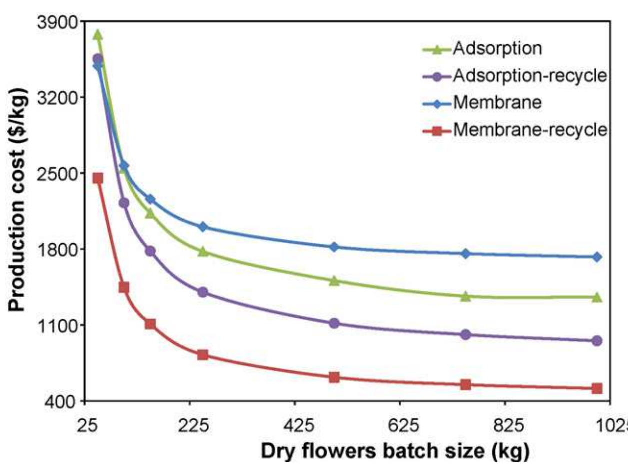

Fig. 5 – Unit production cost of isoflavone rich product as a function of dry flowers batch size.

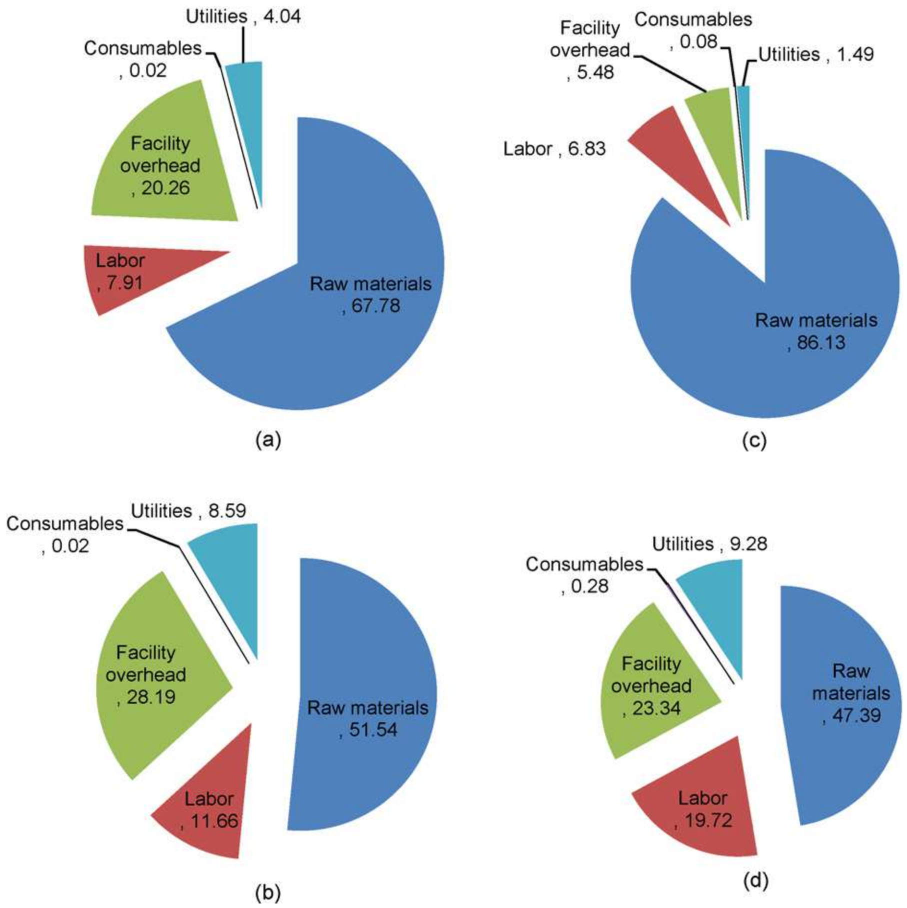

Fig. 6 – Breakdown (%) of annual operating cost for different processes: (a) adsorption; (b) adsorption-recycle; (c) membrane; (d) membrane-recycle.

production cost was considered for further assessment. In accordance with common practice in most process economic evaluations in the public domain, all the economic analysis provided in this work is calculated based on typical US rates, specified in US$. The purchasing cost of $3/kg and $0.75/kg was assumed for dry flowers and ethanol respectively. Also, in order to keep the equipment costs lower, the ultrafiltration modules in the membrane based processes were modeled to be constructed out of plastic, which was appropriate for moderate pressure operations (550 kPa). The processes were evaluated for a 15-year project life time, assuming the plant to be operational for 330 days/year. Furthermore, while the simulator is capable of calculating the waste disposal cost for the process if the treatment cost of output streams is provided; in present analysis any associated waste disposal cost was ignored.

In order to perform the preliminary screening of conceptual flowsheets discussed previously, economic assessment was considered to be adequate. In a batch manufacturing facility, the annual throughput is equal to the batch size times the number of batches that can be processed per year. Increasing the batch size or number of batches per year increases the annual plant throughput and consequently leads to a more economical process.

As shown in Fig. 5, the unit production cost of isoflavone rich product is plotted against different batch sizes for different process scenarios. It can be seen that the production cost is a stronger function of smaller batch sizes. Although, an increment in batch size leads to reduction in the production cost, considering the market demand for isoflavone supplements and any other value added bio-products for that matter, 500 kg of dry flowers per batch could be assumed to be adequate and hence was considered for further evaluation.

The key economic evaluation results for two different standard and modified scenarios are shown in Table 1 in order to compare the economic aspects of different processes. It was worth mentioning that the economic comparison in Table 1 was a representation of computer output for economic evaluation report of SuperPro Designer® and was based on processing of 500 kg dry flowers in a standard 8 hour batch together with material and equipment cost assumptions discussed earlier. Also, it should be noted that for recycle loop and tear streams in modified processes the Wegstein Acceleration was disabled for successive substitution and the relative

Table 1 – Key economic evaluation results.

	Adsorption	Adsorption-recycle	Membrane	Membrane-recycle
Total capital investment (1000$)	15,242	15,356	6713	8000
Operating cost (1000$/year)	12,747	9428	17,176	5843
Total equipment purchase cost (1000$)	2325	2393	849	1228
Main product rate (kg/year)	8447	8447	9443	9443
Product unit cost ($/kg)	1509	1116	1818	618

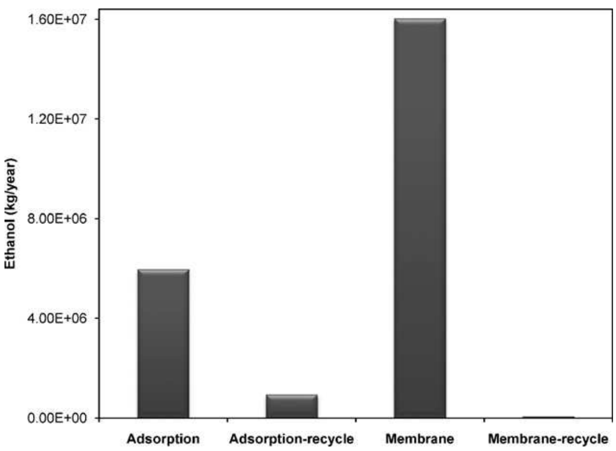

Fig. 7 – Annual ethanol consumption for different isoflavone recovery scenarios.

tolerance for the total flow rate along with temperature convergence was set at 0.001 for 500 maximum iterations as opposed to standard processes, which involved absolute solution. The effects of the variation in the cost of feed and other inputs could be studied by running additional simulations with new values. Therefore, the precision in values listed in Table 1 should not be viewed statistically. Moreover, all the associated costs referred in this study were subjected to an operation located in USA and are expected to change significantly if the production facilities were located elsewhere. It can be observed from Table 1 that in comparison to membrane based processes; the fixed capital investment for a manufacturing facility is higher for adsorption processes due to additional unit procedures involving adsorption and PVP recycling. Moreover, considering the high consumption of ethanol in standard flowsheets, the annual operating cost (assuming the facility is dedicated to a single product) is lower for respective recycle processes. It can be seen that the reduction in unit production cost of isoflavones supplement in case of membrane based processes is significant in comparison with the adsorption processes after the modification. Also, for an identical quantity of raw flowers that are processed, the main product rate for membrane based processes (9443 kg/year) is marginally higher for membrane based process as opposed to adsorption based processes (8447 kg/year). The lowest production cost observed among the four cases discussed was for membrane process with solvent recycle, which was $618/kg. The higher production cost for adsorption based processes can be justified to some extent by higher content of isoflavones (20.8%) in the final product.

Fig. 6 provides the percentage breakdown of annual operating cost of all four processes. Comparing Fig. 6(a) and (b) for adsorption based processes, it can be seen that though the percentage costs for utilities, facility overhead and labor are marginally higher for adsorption-recycle process, the fraction of raw materials cost is much lower in comparison with standard adsorption process. In case of membrane based processes (Fig. 6(c) and (d)), the percentage cost for raw materials is significantly lower except the remaining portions of operating cost, which are considerably higher for membrane-recycle process than that of standard membrane process. The trend can be interpreted as a result of supplementary tray drying operation in membrane-recycle process to facilitate additional solvent recovery from reverse osmosis retentate that further lowers the ethanol consumption. Moreover, it can be seen from Fig. 7 that in addition to overall lowest ethanol consumption, the solvent recycle modification is more effective for membrane-recycle process with almost 99% recycling. One of the reasons for lower recycle efficiency (84%) in the adsorption-recycle process can be due to the limitations of distillation in concentrating recycled ethanol solution to 95% (v/v). Consequently, considering the fact that membrane based processes utilize 50% (v/v) ethanol as a solvent and the major portion of the recovery was achieved by integrating reverse osmosis unit procedure as opposed to distillation unit procedure in adsorption-recycle process, the recycle efficiency in membrane based process was significantly higher.

4. Conclusion

The commercial production of isoflavones supplement using red clover flowers was investigated through the development of four alternate scenarios with a commercial bio-process simulator, provided useful data on economic analysis. Membrane and adsorption based processes with an integration of effluent streams recycling were shown to be feasible. Although the modified recycle processes have higher capital and annual operation costs, the unit production cost of isoflavones concentrate was significantly lower as compared to respective standard processes. Membrane based process with solvent recycle was found to have lowest production cost among the four investigated flowsheets. The production cost for manufacturing isoflavone supplements appears reasonable and could be further optimized by factoring the potential market size. It was shown that by selecting appropriate simulation schemes, the anticipated gain of the processes could be quantified.

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