GOODBYO

MULTI-COMMODITIES MICROBIAL-DRIVEN BIOREFINERY BASED ON FOOD-PROCESSING INDUSTRY WASTES, BIOGENIC CO_2 AND BIOPROCESS WASTEWATERS

Deliverable 5.1 –Systematic and Critical Literature Screening on Separation Processes

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EXECUTIVE SUMMARY

This deliverable (D5.1) of the GoodByO project provides a systematic and critical literature screening focused on state-of-the-art separation processes pertinent to the valorization of food-processing industry wastes, biogenic CO2, and bioprocess wastewaters. It specifically aims to establish a solid foundation for developing robust, efficient, and sustainable separation technologies essential for the multi-commodity biorefinery envisioned by the GoodByO project.

The document initiates by presenting the overall context of biorefinery processes, highlighting the necessity of efficient separation methodologies to recover and purify bio-based products, ensuring economic feasibility, market acceptance, and environmental sustainability. This initial assessment identifies the complexity and challenges inherent in processing feedstocks derived from waste streams, which contain diverse and diluted chemical compositions.

A comprehensive evaluation of existing literature across multiple separation techniques is conducted, with a specific emphasis on the distinct requirements related to microbial-derived commodities targeted in GoodByO, particularly focusing on bio-octanoic acid, bio-hexanol, and microbial biomass recovery.

In-depth coverage is dedicated to the separation of bio-octanoic acid (Task 5.2), critically assessing liquid-liquid extraction methods, reactive extraction, membrane extraction, and crystallization. The screening emphasizes the selection of biocompatible and sustainable solvents, evaluating their extraction efficiencies, selectivities, and integration potential within fermentation processes. The document highlights oleyl alcohol and other renewable solvents for their superior biocompatibility and effectiveness, alongside innovative hybrid extraction techniques combining reactive extraction to enhance product recovery and purity.

Hexanol separation by membranes (Task 5.3) receives significant attention due to the complex and dilute nature of fermentation broths. Membrane-based separation techniques, notably pervaporation, are critically analyzed for their capability in selectively removing bio-hexanol from aqueous mixtures. Pervaporation is highlighted as an energy-efficient solution capable of significantly reducing operational costs, offering high selectivity, and facilitating continuous operation suitable for integration with the gas fermentation processes employed by the GoodByO project.

The recovery of microbial biomass and high-value cellular components (Task 5.4) from fermentation broths is also comprehensively reviewed. Techniques such as ultrafiltration, centrifugation, and cell disruption methods are critically assessed for their efficacy, scalability, and economic viability. Special focus is given to novel approaches and integrated hybrid processes that enhance product purity, yield, and cost-effectiveness, ensuring optimal recovery of valuable microbial products such as proteins, lipids, and carotenoids.

Identifying optimal separation processes is instrumental for validating the economic and environmental sustainability targets of GoodByO. Thus, techno-economic analyses (TEA) and life cycle assessments (LCA) associated with the reviewed technologies are critically assessed to ensure alignment with the project's sustainability and market competitiveness goals.

Overall, Deliverable D5.1 establishes a comprehensive scientific foundation, emphasizing critical separation technologies and strategies necessary for achieving the GoodByO project's ambitious goals in the efficient valorization of industrial biowaste streams, particularly concerning bio-octanoic acid, bio-hexanol, and microbial biomass recovery.

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LIST OF ABBREVIATIONS

C4	Butyric acid
C6	Caproic acid
C8	Caprylic acid
DMF	N,N-dimethylformamide
DMSO	dimethylsulfoxide
DSP	Downstream processing
EG	Ethylene glycol
FFV	Fractional free volume
GPC	Gel permeation chromatography
HRT	hydraulic retention time
HSDB	Hazardous Substances Data Bank
ICP-OES	Inductively Coupled Plasma-Optical Emission Spectrometry
IL	ionic liquid
PIM	Polimer of Intrinsic microporosity
LC-MS	Liquid Chromatography-Mass Spectrometry
LLE	Liquid-Liquid Extraction
M-HRAP	Membrane-coupled high-rate algal ponds
MF	Microfiltration
NF	Nanofiltration
PA	Polyamide
PBO	Polybenzoxazole
PDMS	Polydimethylsiloxane
PI	Polyimide
POMS	Polyoctymethylsiloxane
PSB	Purple sulfur bacteria
PTFE	Polytetrafluoroethylene

PV	Pervaporation
PVA	Polyvinyl alcohol
SBET	Specific surface area
SBI	Spirobisindane
TBP	Tri-n- Butyl Phosphate
TEA	Techno-economic analyses
TEOS	Tetraethoxysilane
TFC	Thin-film composite
TFN	Thin-film nanocomposite
TGA	Thermogravimetric Analysis
UPLC-MS	Ultra-Performance Liquid Chromatography-Mass

1 SURVEY ON CAPRYLIC ACID RECOVER

Caprylic acid (C8), also known as octanoic acid, is an eight-carbon saturated medium-chain fatty acid (MCFA). It is found naturally in the milk of various mammals and is a minor component of coconut oil and palm kernel oil. Historically, caprylic acid has been used in various applications, including as an antimicrobial agent, in the production of esters for perfumes, and in the manufacture of dyes. In the GoodByO project MF1 focuses on caprylic acid production via microbial fermentation, specifically through chain elongation of shorter-chain fatty acids using organic waste streams as feedstocks. This method is conceived to be a sustainable alternative for producing valuable chemicals from renewable resources. Downstream processing (DSP) of caprylic acid, particularly from fermentation broths, is a critical area of research and development to enable cost-effective and efficient production.

1.1 Methods of caprylic acid extraction and purification

The extraction and purification of caprylic acid from fermentation broths present several challenges. The broth is a complex mixture containing water, cells, unreacted substrates, high salt concentrations, various organic acids (including C8), and other metabolites. The relatively low concentration of caprylic acid in the broth, combined with its moderate hydrophobicity and the presence of structurally similar compounds, makes selective extraction difficult. Common methods for C8 extraction and purification include:

- Liquid-Liquid Extraction (LLE): This is the primary focus of the provided text and involves using an organic solvent to selectively extract caprylic acid from the aqueous fermentation broth [1,2]. The success of LLE depends on the choice of solvent, which must meet several criteria (discussed in detail later).
- Reactive Extraction: Use of reactive extractants, that form a complex with the caprylic acid [3].
- Membrane Separation: Membranes can be used to separate the aqueous and organic phases, improving the efficiency of LLE and preventing emulsion formation. They can also be used for other separation techniques, like pervaporation [4,5].
- Distillation (including Vacuum Distillation): This takes advantage of the boiling point difference between caprylic acid and other components of the mixture. Vacuum distillation is often necessary due to the relatively high boiling point of C8 [6].
- Adsorption: Solid adsorbents can be used to selectively bind caprylic acid, followed by desorption to recover the purified product[7].
- Crystallization: This relies on the difference in solubility of caprylic acid or caprylate and other components. Some caprylate salts for instance can be readily precipitated from a fermentation broth [8].

In this short literature study, the focus will be on liquid-liquid extraction as this would allow for in-situ removal of C8 from the fermentation broth. The potential solvents (and possible additives) are to be evaluated on biocompatibility, extraction efficiency, separability, recovery and practical use.

1.2 Fermentative caprylic acid production

The production of MCFA's (including caprylic acid) is done via a two-step process within ChainCraft. During this process, VFA's (e.g. C2, C3, C4) are produced. The effluent of the HAR is the influent of the CER, where the chain elongation happens. Here, caproic acid (C6) is produced from butyric acid (C4) with the addition of ethanol by chain elongation bacteria via the reverse β-oxidation pathway.[9,10] C6 can be further elongated further to C8 using the same process.[9] See Equation 1-3 for the specific reactions. Overall, 1 mole of acetate and 3 moles of ethanol are used to produce 1 mole of caprylate and 3 moles of water, see Equation 4. Equations 1-4 are a simplified representation of the process.

$C_2H_3O_2^- + C_2H_5OH \rightarrow C_4H_7O_2^- + H_2O$ (1) Acetate to Butyrate

$C_4H_7O_2^- + C_2H_5OH \rightarrow C_6H_{11}O_2^- + H_2O$ (2) Butyrate to Caproate

$C_6H_{11}O_2^- + C_2H_5OH \rightarrow C_8H_{15}O_2^- + H_2O$ (3) Caproate to Caprylate

$C_2H_3O_2^- + 3\,C_2H_5OH \rightarrow C_8H_{15}O_2^- + 3\,H_2O$ (4) Overall stoichiometric reaction

The C8 from the chain elongation reactor will be extracted by using an organic solvent with a continuous in-situ method. In anaerobic C8 production, product inhibition significantly hinders fast production. This inhibition is caused by two reasons. 1) Product inhibition: Caprylic acid is toxic to bacteria at relatively low concentrations. @ref Removing it reduces this toxicity 2) Thermodynamics: the biological formation of C8 is in equilibrium with the production C4 and C6. When C8 is extracted out, the equilibrium of biological product formation will shift more towards the formation of C8.

1.3 Solvent criteria

Organic solvents will be used as an extractant solvent to extract C8 out of aqueous phases using liquid-liquid extraction. These solvents are required to have properties crucial to the extraction process that will later be integrated into the production process, see Table I for a summary of solvent criteria with their attached parameters. The solvent must be biocompatible, if the solvent is toxic to chain elongating bacteria, it will be unusable immediately. It must also not inhibit the production of C8 or be broken down itself, to a degree that impairs economically feasible application. Extraction efficiency is a broad term and describes how well the solvent can selectively extract C8. The solvent needs to be able to extract C8 to a reasonable degree and not extract too many of other unwanted compounds. Moreover, separability is important because it defines how well the two-phasic system forms. For example, solvent must not dissolve too much into the aqueous phase and not form an emulsion with the reactor broth.

Table I. Solvent criteria, with their parameters, if it is a hard or soft parameter and the motivation and impact of the parameters.

• **Biocompatibility:**
○ **Biological Toxicity:** The solvent *must* be non-toxic to the chain-elongating bacteria. This is a "hard" criterion; a toxic solvent would halt production.
○ **Chain Elongation Inhibition:** Ideally, the solvent should not inhibit the chain elongation process itself, even if it's not directly toxic. This is a "soft" criterion, as some inhibition might be acceptable if outweighed by other benefits.
○ **Biological Degradability:** The solvent should preferably *not* be readily biodegradable by the microorganisms in the reactor, as this would lead to solvent loss and potentially complicate the process.
• **Extraction Efficiency:**
○ **Partition Coefficient of C8:** This is a critical "hard" criterion. A high P_{C8} indicates that C8 will preferentially partition into the solvent, leading to efficient extraction.
○ **Partition Coefficient of Other Compounds:** This is also a "hard" criterion. Low partition coefficients for other components of the fermentation broth are desirable to ensure selective C8 extraction.
○ **Extraction Selectivity for C8:** This "soft" criterion reflects the ratio of P_{C8} to the partition coefficients of other compounds. High selectivity simplifies downstream purification. However, some co-extraction of for instance C6 might be acceptable.
○ **Time for Equilibrium:** A shorter time to reach equilibrium between the aqueous and organic phases is preferred for faster extraction. Largely dependent on viscosity, maximum solubility, phase separation/emulsifying properties and mixing regimes.
• **Separability:**
○ **Time to Form Two-Phasic System:** Rapid phase separation is crucial for efficient continuous extraction. This is a "soft" criterion, but slow separation can significantly impact process efficiency and could necessitate active phase-separation processes (e.g. gravitational methods).
○ **Solubility in Fermentation Broth:** Low solvent solubility in the aqueous phase is a "hard" criterion (especially without a membrane). Solvent loss into the broth can contaminate the product, inhibit the microorganisms, and complicate downstream processing.
○ **Degree of Emulsification:** Minimal emulsion formation is essential for easy phase separation. This is a "hard" criterion, as stable emulsions can make separation extremely difficult.
• **Recovery from Solvent:**
○ **Ease of C8 Back-Extraction from Solvent:** This refers to the ease of recovering the extracted C8 from the solvent (e.g. via back-extraction or vacuum distillation). While initially a "soft" criterion, it's crucial for overall process viability.
○ **Recyclability/Chemical Stability:** Refers to the ability of the solvent to be recycled and reused after back-extraction, as well as the resistence to chemical change during the process.
• **Practical Use:**
○ **Viscosity:** Low viscosity is preferred for easier pumping and better mass transfer.
○ **Analytical Measurability:** The solvent should ideally not interfere with the analytical methods used to quantify C8 and other components in the fermentation broth.
○ **Safety:** The solvent must be safe to handle in both laboratory and industrial settings (low flammability, low toxicity, etc.).

○ **Density:** A significant density difference between the solvent and the aqueous phase facilitates phase separation.
○ **Ecological Viability:** The use of renewable solvents or solvents with minimal environmental impact is preferred.

1.4 Candidate solvents for in situ caprylic acid extraction

Mineral oil

Mineral oil is a viscous mixture of long chain alkanes mainly C15–C50 hydrocarbons and high boiling point hydrocarbons, derived from petroleum.[11] The viscosity can vary greatly, but is usually between 80-100 mPa·s.[12] Its boiling point generally lies above 300°C and individual fractions can go up to 600°C. [13–16] Its density is lower than water, generally around 0,83-0,86 g/ml for light versions and 0,875-0,905 g/ml for heavy versions, light mineral oil is less viscous than heavy mineral oil.[16,17] Mineral oil is insoluble in water, but extraction with this solvent in pair with fermentative technologies is usually done with a membrane. [4,18–20] There are no articles particularly describing the toxicity of mineral oil on microbes. If this solvent can be reused multiple times (high recyclability), it may be a good option, despite being a petroleum product.

Oleyl alcohol

Oleyl alcohol is an 18 carbon chain fatty alcohol derived from oleic acid (see **Error! R eference source not found.**), which is extracted from olive oil, meaning it is a renewable resource.[21] Research shows that oleyl alcohol has a high affinity for C8 and has a LogP value of 2,28 at pH 4,8. [22] Its boiling point is around 305-385°C, depending on purity.[21,23,24] Its density is also lower than water, which is around 0,85 g/ml.[24] It has a viscosity of 28,32 mPa·s.[25] Oleyl alcohol is insoluble in water. [23] This solvent is most likely not toxic to bacteria in an in-situ extraction setup, as it has been used in literature for this purpose before.[26]

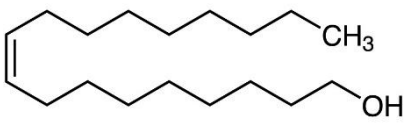


Figure 1. Chemical structure of oleyl alcohol

Sunflower oil

Vegetable oil, in particular sunflower oil might be able to extract C8 from the CER broth. Research has been done with sunflower oil and other vegetable oils in extracting caproic acid from fermentation mediums with the use of reactive extractants.[27,28] Sunflower oil is primarily made up of linoleic (Figure 2) and oleic (Figure 3) fatty acids in a triglyceride form.[29] The smoke point of sunflower oil is around 230-250°C.[30,31] This is the temperature

the oil starts to break down into free fatty acids and glycerol. Its density is around 0,92 g/ml.[32] Its viscosity is 48,8 mPa·s.[33] Sunflower oil is insoluble in water but free fatty acids in the sunflower oil mixture might be soluble in water. Unsaturated fatty acid groups might also become a target for chemical conversions. Sunflower oil is not toxic to bacteria. A problem that might arise if this solvent is used as the extractant solvent in the in-situ system, is that it might biodegrade because of microbial digestion.[34]

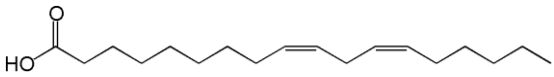


Figure 2. Chemical structure of linoleic acid

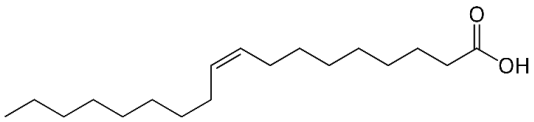


Figure 3. Chemical structure of oleic acid

Hexane

Hexane is the final solvent to be used in this research, it is a 6-carbon chain alkane it is a widely used extractant solvent in laboratory and industrial use.[35] This solvent is highly flammable and has a negative effect on the environment and human health.[36] It has been used before to extract C6 and C8 out of a fermentation medium.[37] It has a viscosity of 0,297 mPa·s.[33] With a boiling point of 69°C it has a significantly lower boiling point than the other solvents.[38] The same can be said for its density; being 0,659 g/ml.[38] Hexane is insoluble in water. Hexane supposedly is not toxic to anaerobic bacteria in a saturated aqueous phase.[39] Even though hexane is a byproduct of the petroleum industry, if it can be reused multiple times, it may still prove to be a viable candidate.

Octanol

Octanol, specifically 1-octanol, is an eight-carbon chain alcohol. It is a common solvent used in various industrial applications. Its chemical formula is $CH_3(CH_2)_7OH$. Octanol has a moderate boiling point of around 195°C. Its density is approximately 0.824 g/mL, making it less dense than water. The viscosity of octanol is around 7.2 mPa·s. Octanol is only slightly soluble in water, with a solubility of approximately 0.05% at room temperature. It has a LogP value of around 3, indicating a preference for the organic phase. While octanol can be toxic to some organisms at high concentrations, it is often used in biphasic systems with microorganisms and can be tolerated at lower concentrations. Its use in extraction processes with fermentation broths has been documented. Like other alcohols, it is biodegradable, which could be a factor to consider in long-term applications.

Decanol

Decanol, also known as decyl alcohol or 1-decanol, is a ten-carbon chain primary alcohol. Its chemical formula is $CH_3(CH_2)_9OH$. Decanol has a higher boiling point than octanol, around 230°C. Its density is approximately 0.835 g/mL. The viscosity of decanol is higher than octanol, around 12.5 mPa·s. Decanol's solubility in water is even lower than octanol, making it practically insoluble. It has a higher LogP value than octanol, indicating an even stronger preference for the organic phase. Similar to octanol, decanol can exhibit some toxicity to microorganisms at higher concentrations, but it can be used in biphasic systems at appropriate concentrations. Decanol has also been explored as an extractant in fermentation processes. Its biodegradability is a factor to consider, similar to octanol.

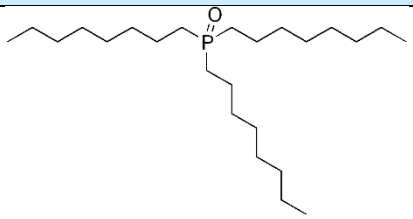
1.5 Reactive extractants

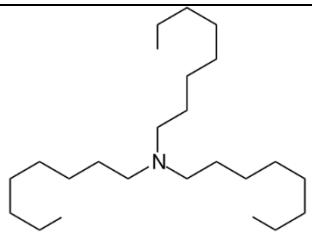
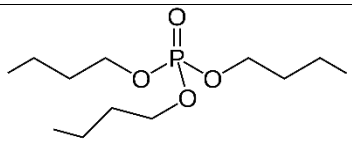
Reactive extractants can be added to the solvent to aid in the extraction of carboxylic acids. The reactive extractants acts as complexing agents with carboxylic acids in the organic phase. This way the maximum solubility of the carboxylic acids in the solvent side can be increased, allowing for a higher capacity and driving force.[40] The reactive extractants are generally toxic to bacteria, which could present a problem to in-situ extraction systems in cases where no membranes are used to keep the phases separate.[41] Possibly this challenge can be overcome by further diluting the reactive extractant in the solvent and/or having the actives cells immobilized in for instance biofilm systems.[41]

Two types of complexing reactants that are usually used to recover carboxylic acids from fermentation broths are high-molecular-weight aliphatic amines and organophosphorus compounds, such as (Table II) tri-n-octylphosphine oxide (TOPO), trioctylamine (TOA) and tributyl phosphate (TBD). All these compounds have been widely used in literature as a reactive extractant.[18,41–43] In

Table II their solubily in water, their boiling point, general safety aspect and molecular structure are shown.[44–46]

Table II. Reactive extractants and some of their properties

	Property			
Reactive extractant	Solubility in water (g/L)	Boiling point (°C)	Safety	Molecular Structure
TOPO	Insoluble	212-213	Very toxic to water organisms, irritating.	

TOA	Insoluble	365-367	Toxic to aquatic organisms, irritating	
TBD	>0,3g/L	288	Irritating	

In situ extraction systems

The Liquid-liquid extraction can be performed via various integrated setups. In previous research on in situ extraction techniques the various solvents were evaluated, including some with membrane usage[18,27,37,47]. However, the extraction efficiency of these solvents were not always reported. These will have to be evaluated during the GoodByO project. Moreover, the effect of the complex fermentation broth on solvent-aqueous phase separability will need to be thoroughly evaluated.

For instance, present ions will affect the ionic strength and an elicit various buffer capacities, affecting acid dissociation constants of the fatty acids and in turn the partitioning. In the case of acetic acid, the pKa value is higher at a higher ionic strength.[48] In turn making more acid protonated at a higher pH's, which is favourable for the extraction. It is speculated this is the same case for the other fatty acids. Extraction at a pH where most of the fatty acids are protonated is preferable, as it would lead to a higher extraction into the organic phase. Literature shows that fermentative production of C8 using ethanol is usually done at pH ~5,5.[4,18,49] With a pKa of ~4,89 most of the C8 will be deprotonated in this environment.

The in-situ extraction systems can be done internally or externally. With an internal in-situ extraction setup the organic solvent is in direct contact with the fermentation broth inside the bioreactor tank, whereas with external extraction the broth recirculated over an external unit (see Figure 4).[3] More detailed depictions of possible configurations are given in Figure 5. Within GoodByO the focus will likely be on semi-partitioning bioreactors for the lab research, and multi-phase bioreactors for the pilot research (Figure 5)[3]. DAB.io bio provides inspiration for these designs as they have developed a dedicated semi-partitioning module called ISPRIN for lab research, as well as an upscale multi-phase bioreactor design called FAST™ [50] (Figure 6). The ISPRIN works by inserting a hollow tube that functions as a settler in the fermenter with an opening for fluid exchange.[51] The organic phase with fermentation product(s) settles in this hollow tube where it is pumped to another container to collect or back extract the product. Critical to be evaluated will be the shear stress tolerance of the microorganisms and the impact of such modules design on the bioprocess sterility. All the above listed aspects will need to be balanced for the design of a proper solution to allow reaching the project objective. For the latter, more details will be reported in the front end engineering design.

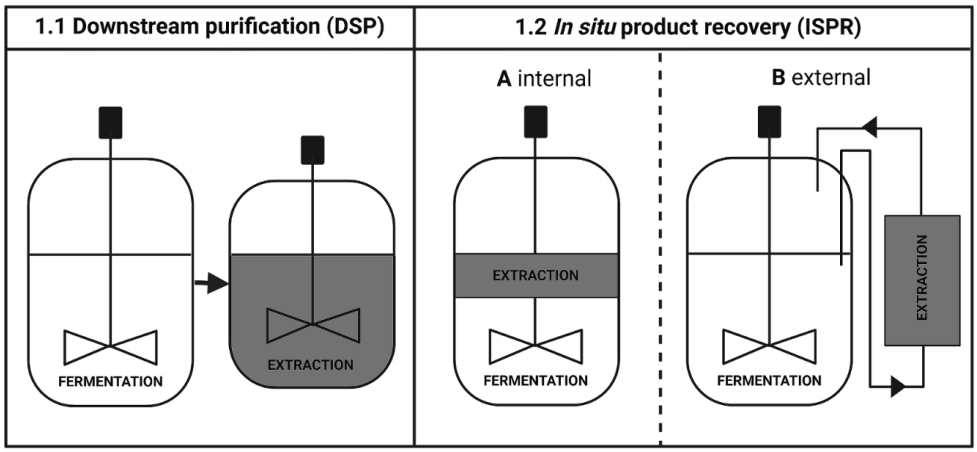


Figure 4. Schematic depictions of internal and external in-situ extraction setup, taken from Tönjes et al. (2025).

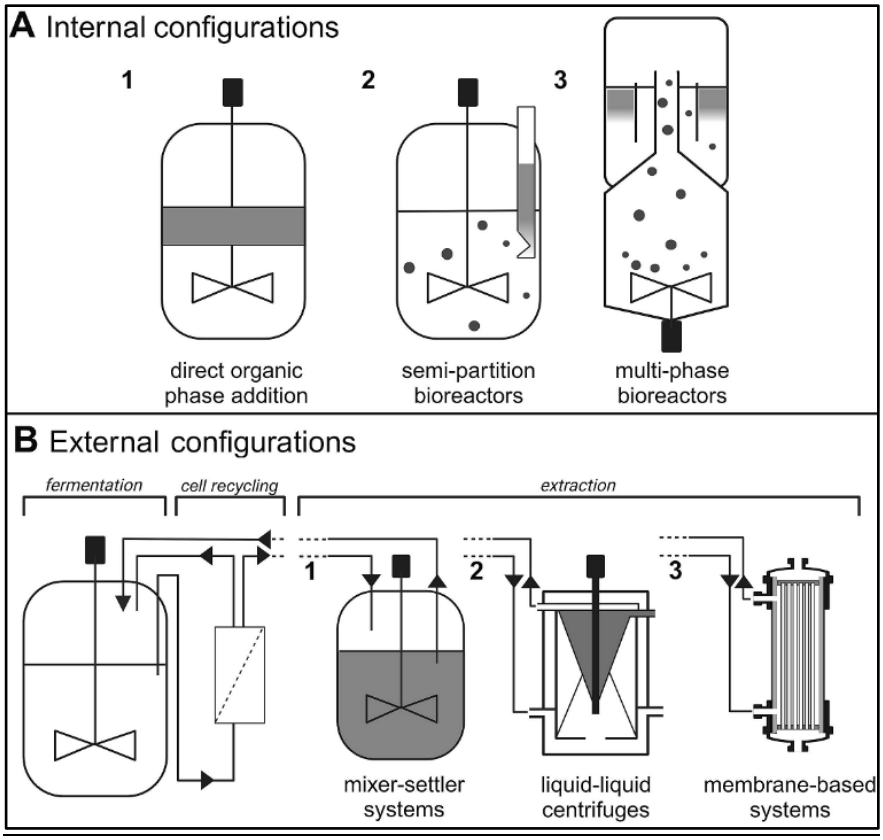


Figure 5. Schematic view of various internal and external in-situ extraction setups. Figure taken from Tönjes et al. (2025)

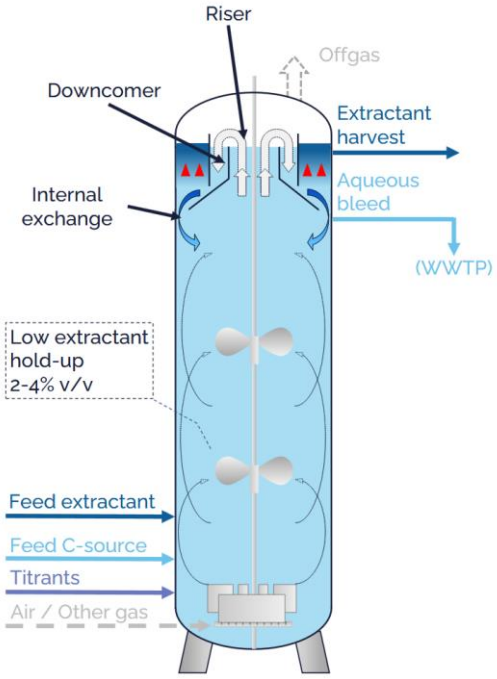


Figure 6. Schematic view DAB.bio FAST™ fermenter concept with and external in-situ extraction. Figure taken from FAST™ Technology: Technical Flyer.

1.6 Back extraction & recovery

Vacuum distillation uses reduced pressure to purify a product stream. This method enables the purification of compounds that are difficult to distil at ambient pressures, or it can be used to save time and energy. Caprylic acid has a boiling point of 239°C which makes the use of vacuum distillation appealing.[52] This method allows for high purity fatty acid fractions, free of water as these are separated by temperature.[53] The downside of this method is the high energy requirements for the distillation.

An alkaline back extraction can be used to extract the fatty acids back into an aqueous phase again.[54] An alkaline solution in contact with the (previously separated) organic phase results in the release of the dissociated acid in its salt form to the aqueous phase. The largest downside of this technique is the high requirement of bases, and the recovery of organic acids will be in their salt form and still within an aqueous phase. This technique also does not selectively extract out a particular fatty acid and DSP is needed to separate them. This approach might be a more viable option in a laboratory setting in case solvent regeneration is required, due to its more practical applicability. However, industrial application would depend fully on the follow-up process steps and how the carboxylate salts and then further processed.

1.7 Concluding remarks

Additives could enhance liquid-liquid extraction via forms of reactive extraction, as long as they are not found to interfere with the biological activity. This is to be assessed. Membrane separation is to be avoided, as the introduction of membrane would complicate and drive up the costs of upscaling the selected in-situ fermenter design. Since the goal is to work with large complex organic waste streams in large reactors for bulk chemical production, the process needs to be developed as simple as possible.

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2 1-HEXANOL EXTRACTION USING PERVAPORATION

2.1 Membrane Pervaporation.

2.1.1. Background.

The environmental footprint of the life cycle associated with organic solvents can be minimized by recovering and reusing these solvents, thereby eliminating the need for producing new solvents and managing the disposal of used ones. The reuse or reprocessing of solvents necessitates the use of separation methods to extract and purify the solvents from mixtures formed during industrial processes.[1] In this field, industrial membranes have established themselves in recent years as essential components of chemical processing industries, to the point that membrane-based technology is currently regarded as a new frontier of chemical engineering.[2]

Pervaporation is a recent membrane-based separation technique that shares certain features with reverse osmosis and gas membrane separation. In pervaporation, the liquid mixture to be separated (feed) contacts one side of a membrane, while the permeated product (permeate) is extracted as a low-pressure vapor from the opposite side (Figure 7). The driving force for transport is the difference in chemical potential between the feed-side mixture and the permeate, typically expressed in terms of fugacity or, more practically, the partial pressure of each compound. The vapor pressure difference can be sustained through various methods. In a laboratory setting, a vacuum pump is employed to create a vacuum on the permeate side of the system. In industrial applications, the most cost-effective way to generate a vacuum is by cooling the permeate vapor, which induces condensation; this condensation naturally generates a partial vacuum.

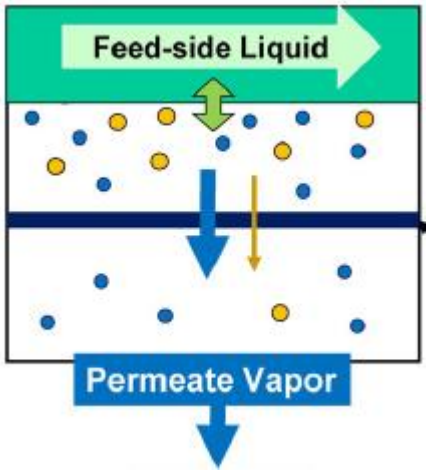

Figure 7. Schematic diagram of the pervaporation process.[1]

Although pervaporation is among the most prominent topics in contemporary membrane research, the idea of pervaporation-based separation is not a recent one. The concept of pervaporation dates to the 19th century, when Kober (1917) first observed this phenomenon, coining the term in a publication reporting selective permeation of water from aqueous solutions of albumin and toluene through collodion (cellulose nitrate) films.[3] The process was first studied in a systematic manner by Binning and coworkers at

American Oil in the 1950s, due to the interest in applying the process to the separation of organic mixtures.[4] The process became competitive. Research on it was resumed in the 1970s by Eli Perry and colleagues at Monsanto where, between 1973 and 1980, more than twelve patents were granted, covering a broad range of pervaporation applications, though none of this research resulted in a commercial process. Academic studies on pervaporation were also conducted by Aptel, Neel, and others at the University of Toulouse.[5,6] A major advancement occurred in the early 1980s, when gesellschaft für Trenntechnik (GFT) Co. developed a composite membrane consisting of a thin layer of crosslinked poly(vinyl alcohol) supported by a porous poly(acrylonitrile) substrate. A pervaporation process for ethanol dehydration was then commercialized. In the following years, significant research expanded the scope to include various liquid mixtures and several types of membranes. Membranes made from both synthetic and natural polymers derivatives have been tested for separating various liquid mixtures, including, for example, alcohol-water, acetone-water, methanol/methyl tert-butyl ether, methanol/pentane, toluene-heptane, and isomeric xylenes.[7]

Pervaporation applications can be divided into three main categories: (i) dehydration of organic solvents, (ii) extraction of organic compounds from aqueous solutions, and (iii) separation of anhydrous organic mixtures. At present, pervaporation has been commercialized for two primary uses: one is the dehydration of alcohols and other solvents, while the other is the removal of trace organic compounds from polluted water. In the latter case, pollution control and solvent recovery are achieved simultaneously. Additional promising applications include aroma recovery and beer de-alcoholization in the food industry, as well as product recovery from fermentation broths to enhance bioconversion processes.[8] Pilot plants for separating organic/organic mixtures have also been constructed by Separex[9] and W.R. Grace[10].

2.1.2. Fundamentals of Pervaporation Process.

The separation in pervaporation is determined by the chemical characteristics of the macromolecules forming the membrane, the membrane's physical configuration, the physicochemical attributes of the mixtures to be separated, as well as the interactions between permeants and between permeants and the membrane.
To investigate the factors influencing the performance of pervaporation process, it is essential to identify the relationships and parameters that characterize membrane performance.

2.1.2.1. Solution-diffusion Model.

A comprehensive understanding of mass transfer phenomena is crucial for researchers to identify optimal membrane materials and develop the appropriate structure and configuration of membranes. Böddeker[11] has provided an overview of the key concepts and terminology associated with pervaporation. The permeation of a component through a pervaporation membrane can be analysed from both thermodynamic and kinetic perspectives. From a thermodynamic viewpoint, it involves the solubility of the

component within the membrane material, while from a kinetic standpoint, it concerns the diffusion of the penetrant through the membrane. The transport interactions among various penetrants also influence the permeation of each individual component. Furthermore, these penetrants may cause the membrane to swell, alter its microscopic structure, and significantly increase diffusion rates compared to those observed in a dry membrane.[4,12]

The solution-diffusion model is the most accepted mechanism for describing transport in pervaporation. According to this model, the movement of a permeating component occurs in three sequential steps (Figure 8):

1. Sorption of the permeating component from the feed liquid into the membrane;
2. Diffusion of the permeating component across the membrane;
3. Desorption of the permeating component into the vapor phase on the membrane's downstream side.

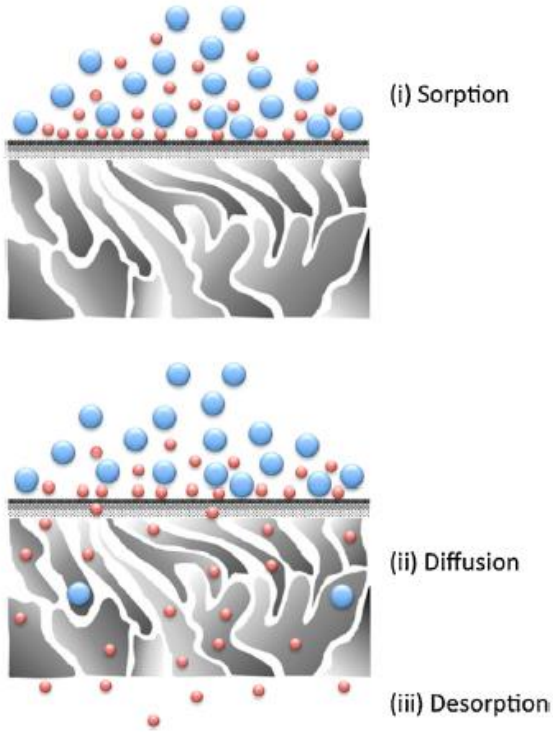

Figure 8. Graphical representation of the solution-diffusion model.[13]

The sorption selectivity of membranes tends to favor molecules that are more condensable or those that exhibit specific interactions with the membrane material. Typically, the closer the solubility parameters of a membrane material and a solute are, the greater the preferential sorption of the solute into the membrane.

By contrast, diffusion selectivity largely depends on factors such as the size and shape of the permeating molecules, the mobility of the polymer chains, the interstitial spaces between them, and the interactions both among the permeating components and between these components and the membrane material.[14]

2.1.2.1.1. Concentration and Pressure Gradients in Membranes.

The mathematical description of diffusion in membranes begins with the thermodynamic principle that the driving forces of pressure, temperature, concentration, and electrical potential are interconnected. The overall driving force causing the movement of a permeant is, therefore, a gradient in its chemical potential. Consequently, the performance of a pervaporation membrane for a specific feed composition and temperature is typically expressed using metrics such as total mass flux or water mass flux, along with either the separation factor of species 1 relative to species 2 (β_{12}), or the purity of the permeate in terms of water weight fraction (w_w^P). The mass flux of a species (J_i, measured in kg $m^{-2}s^{-1}$) is defined as the mass of the species (m_i, in kilograms) that permeated the membrane per unit area (A, in m^2) and unit time (t, in seconds), as:

$$J_i = \frac{m_i}{A\,t}$$

Equation 1.

The molar flux (j_i, measured in kmol $m^{-2}s^{-1}$) is the mass flux divided by the molecular weight (M_i, in kg $kmol^{-1}$).

The molar flux of a compound through a permselective membrane depends on the transport properties of the membrane and the partial vapor pressure gradient driving the transport, which is represented as[15]:

$$J_i = \frac{P_i}{l}\left(p_i^F - p_i^P\right)$$

Equation 2.

where P_i is the molar permeability of species *i* in the permselective material (in kmol m $m^{-2}s^{-1}kPa^{-1}$), *l* is the thickness of the permselective layer (in meters), and p_i^F and p_i^p are the partial pressures (in kPa) of species *i* in the feed-side fluid and permeate vapor, respectively.

Permeability is defined as the product of the solubility and diffusivity of a mobile species within the dense permselective membrane. For a membrane with a fixed or unknown selective layer thickness, the ratio P_i/l, referred to as *permeance* (measured in kmol $m^{-2}s^{-1}kPa^{-1}$), is often the most suitable parameter for evaluating performance.

The separation factor for pervaporation is defined in a manner like relative volatility, as the ratio of the permeate vapor compositions compared to the ratio of the feed-side compositions for two species[15]:

$$\beta_{12} = \frac{(w_1^P/w_2^P)}{(w_1^F/w_2^F)} = \frac{(y_1/y_2)}{(x_1/x_2)} = \frac{(j_1/j_2)}{(x_1/x_2)}$$

Equation 3.

where w_i^k is the mass fraction of species i in stream k [feed (F), retentate I, or permeate (P)], respectively, and x_i and y_i are the mole fractions in the feed fluid and permeate vapor, respectively.

The ratio of the permeate mass fractions is equal to the ratio of mass fluxes, and as demonstrated in Equation 3, the ratio of permeate mole fractions in equivalent to the ratio of molar fluxes. Therefore, with the feed composition known, the separation factor can be determined from the permeate water purity and vice versa. Similarly, given the permeate composition, the total flux can be calculated from the water flux and vice versa.[1]

In some instances, the "enrichment factor" is uses to describe the concentration ratio of the preferentially permeating component between the permeate and the feed. On the other hand, the membrane's intrinsic separation efficiency is defined by its selectivity (α), which represents the ratio of the permeabilities of components i and j. Selectivity can be further categorised into mass-based selectivity and mole-based selectivity[11,16]:

$$(\alpha_{ij})_{mass} = \left(\frac{P_i}{P_j}\right)_{mass}$$

Equation 4.

$$(\alpha_{ij})_{mole} = \left(\frac{M_j}{M_i}\right)_{mass} \mathbf{x} \left(\frac{P_i}{P_j}\right)_{mass}$$

Equation 5.

Mole-based selectivity eliminates the influence of the molar masses (M) of components i and j in the calculation[13].

2.1.2.2. Boundary Layer Effect: Concentration Polarization.

In membrane separation processes, a gas or liquid mixture meets the feed side of the membrane, while a permeate enriched in one of the mixture's components is collected from the downstream side. Since the components of the feed mixture permeate the membrane at different rates, concentration gradients can develop in the fluids on both sides of the membrane. As a result, the concentrations at the membrane surfaces differ from those in the bulk fluid. This alters the permeation behaviour through the membrane, a phenomenon known as *concentration polarization*[17] (Figure 9). The importance of concentration polarization is related to the membrane separation process, as this phenomenon can significantly affect the membrane's performance.

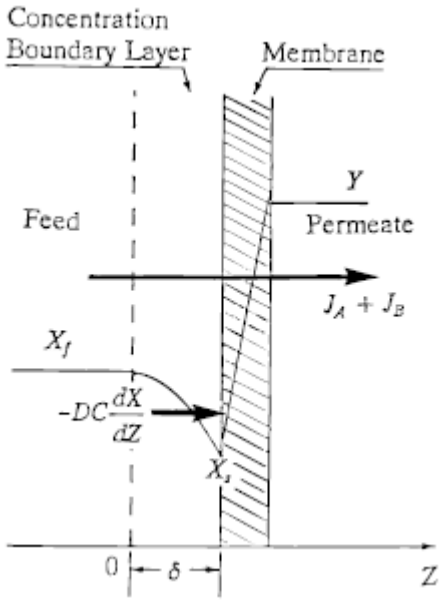

Figure 9. Schematic diagram of the boundary layer effect: concentration polarization.[2]

Two methods have been employed to describe the impact of concentration polarization. The first one is rooted in dimensional analysis, commonly applied to solve heat transfer problems. This approach considers the resistance to permeation through the membrane and the resistance within the fluid layers adjacent to the membrane as a series of resistances.

The second approach to describing concentration polarization involves modelling the phenomenon by assuming the presence of a thin and unmixed fluid layer of thickness d between the membrane surface and the well-mixed bulk solution. Within this layer, concentration gradients develop, driving concentration polarization. Although the boundary layer film model simplifies the complex fluid dynamics occurring in membrane modules and relies on an adjustable parameter – the boundary layer thickness – it is still capable of explaining most experimental data.

2.1.2.2.1. Resistance-in-series Model.

The resistance-in-series model is the primary framework used to describe concentration polarization. According to this model, the overall mas transfer resistance (Equation 6) is the sum of the membrane resistance and the resistance of the liquid boundary layer.

$$\frac{1}{k_t} = \frac{1}{k_l} + \frac{1}{k_m}$$

Equation 6.

where k_t, k_m, and k_l are the overall mass-transfer coefficient and the mass-transfer coefficients in the membrane and the boundary layer, respectively.

When the mass-transfer coefficient of the fluid layer (k_l) is high, the resistance of this layer ($1/k_l$) is low, meaning the overall resistance is primarily determined by the membrane. Conversely, when the mass transfer coefficient is low, the resistance term ($1/k_l$) becomes significant, constituting a substantial portion of the total resistance to

permeation. As a result, the overall mass-transfer coefficient ($1/k_t$) decreases, leading to a reduction in flux. Thus, the boundary layer mass transfer coefficient serves as a numerical adjustment to account for the impact of concentration polarization on the membrane permeation rate.[17]
However, this model is valid only for the most permeable component, whose concentration gradient in the boundary layer is in the same direction as mass transport. For that reason, it has found applicability in pervaporation separation of dilute organic compounds in aqueous solutions, where the concentration polarization has little effect on the permeation rate of water.[2]

An alternative approach shows that the importance of concentration polarization is influenced not only by the membrane's permeability and flow conditions, but also by its selectivity, as described by the following Equation 7:

$$\frac{X_S}{X_F} = \frac{1}{\beta - (\beta - 1)^{-\nu/k}}$$

Equation 7.

where X_S/X_F is the ratio of the mole fraction of the fast-permeating species on the membrane surface and in the bulk feed, β is the "intrinsic" enrichment factor of the membrane based on the concentration that the membrane "sees", and ν and k are the overall molar average velocity of mass transport and the mass-transfer coefficient in the boundary layer. The ratio ν/k is a measure of mass-transfer resistance of the boundary layer relative that of the membrane.
The Equation 7 shows that concentration polarization is intensified with an increase in membrane permeability and selectivity, and a decrease in the mass-transfer coefficient in the boundary layer. Furthermore, when ν/k is sufficiently small, X_S/X_F approaches to unity, which means that concentration polarization is negligible.
In practice, based on the calculated X_S/X_F value, the severity of the boundary layer effect can be assessed. Since controlling the diffusion coefficient in the boundary layer is challenging, the practical solution is to enhance fluid mixing near the membrane surface to reduce boundary layer thickness, which, in turn, increases k and minimizes concentration polarization.

2.1.3. Selection of Polymer Membrane Components.

Polymeric materials are widely used in pervaporation membranes, with material selection often based on empirical methods. Polymers with high selectivity are often prioritized for further research, as their low permeability can be partially mitigated by incorporating asymmetry into the membrane structure, which reduces the effective membrane thickness. Various strategies for selecting membrane materials have been explored, and the main ones are discussed below.

2.1.3.1. Solubility Parameter Approach.

The solubility aspect of pervaporation is emphasized in membrane material selection, based on the idea that preferential sorption is key to preferential permeation. The solubility of a component in the membrane depends on the chemical properties of the material and the permeant, described by their solubility parameters. For a permeant A and a membrane M, higher mutual solubility corresponds to a smaller difference in their solubility parameters. Thus, the ratio of solubility parameters differences (Δ_{AM}/Δ_{BM}) is used as an index for selecting membranes, aiming to favour or reject specific permeants.[2]

However, this approach has limitations. First, preferential sorption does not necessarily result in preferential permeation, as differences in diffusivity also influence – and in some cases dictate – permeation selectivity. The selectivity (α_{mem}) of pervaporation membranes plays a crucial role in determining the overall separation performance and it is strongly influenced by the choice of membrane material. For this reason, membrane materials are specifically designed to address separation challenges.[18]

According to the solution-diffusion model, the membrane selectivity term (α_{mem}) can be written as:

$$\alpha_{mem} = \left(\frac{D_i}{D_j}\right)\left(\frac{K_i^G}{K_j^G}\right)$$

Equation 8.

This equation (8) indicates that membrane selectivity is the result of two factors: the mobility selectivity (D_i/D_j), which is typically determined by the relative mobility of the permeants, and the solubility selectivity (K_i^G/K_j^G), which is primarily influenced by the chemical properties of the membrane material. Experimentally, it has been observed that if $(K_i^G/K_j^G)>1$ and $(D_i/D_j)<1$, or if $(K_i^G/K_j^G)<1$ and $(D_i/D_j)>1$, the membrane demonstrates opposite selectivity for sorption and diffusion; therefore, preferential permeation id dictated by whether sorption selectivity or diffusion selectivity predominate. Hence, the solubility parameter method can be misleading in scenarios where selective diffusion plays the dominant role in separation.

Furthermore, the solubility parameter fails to fully account for complex interactions in a ternary system, and variability in solubility values introduces uncertainty in predictions. Because of this limitations, the solubility parameter method is practical as an initial guideline for choosing polymer membrane materials.[2]

2.1.3.2. Surface Thermodynamics Approach.

This method involves two interfacial free-energy parameters, ΔF_{123} and ΔF_{132}, representing the surface energy between water and the membrane in presence of an organic compound, and between water and the organic compound in presence of the membrane. These values are determined using interfacial tension data from existing studies. Based on surface thermodynamics, a negative ΔF_{123} indicates that the organic compound is preferentially absorbed into the polymer, while a high ΔF_{132} value signifies effective separation of the organic compound from water by the polymer.[2]

However, since this approach focuses on interfacial properties and neglects diffusion (i.e. the kinetic factor), it cannot predict which component preferentially permeates. Additionally, it shares similar drawbacks to the solubility parameter method.[2]

2.1.4. Activation Energy in Pervaporation Processes.

In contrast to reverse osmosis and membrane gas separation, pervaporation involves a phase transition of the permeating species from the liquid state to the vapor state. As a result, energy is required to vaporize the permeate.
According to the solution-diffusion model, the temperature dependence of the permeation flux can be expressed phenomenologically as follows:

$$J_i = \frac{P_i}{l}(p_{i0} - p_{i1})$$

Equation 9.

where p_0 and p_1 are the partial vapor pressure of the permeant in the liquid feed and vapor permeate, respectively, and l is the membrane thickness. P, representing the permeability coefficient of the membrane, is related to the solubility coefficient (S) and diffusivity coefficient (D):

$$P = DS$$

Equation 10.

where D and S depend on temperature according to the following relationships:

$$D = D_0^{(-E_D/RT)}$$

Equation 11.

$$S = S_0^{(-\Delta H_S/RT)}$$

Equation 12.

Thus, the following relation results:

$$P = P_0^{\{(-\frac{E_P}{RT})\}}$$

Equation 13.

where $E_P(=E_D+\Delta H_S)$ is the activation energy of the permeation, which is a combination of the activation energy of diffusion (E_D) and the enthalpy of dissolution (ΔH_S) of the permeant in the membrane.[2]
Rearranging equations 9 and 13 yields membrane permeance (P/l), which is more useful:

$$P/l = J/\Delta_P = (P_0/l)^{-E_P/RT}$$

Equation 14.

where Δ_P is the transmembrane partial pressure difference. Since temperature affects both the membrane permeability and the driving force for mass transport, the activation energy (E_P), representing the temperature dependence of the membrane's permeability coefficient, should be determined from the slope of the $\ln(J/\Delta_P)$ vs 1/T plot. The E_J value

obtained from the lnJ vs 1/T plot is a composite parameter that reflects the overall temperature dependence of the permeation flux.
When the permeate pressure is much lower than the vapor pressure of the feed liquid, the temperature dependence of the permeability coefficient should be determined using the simplified following equation:

$$E_P = E_J - \Delta H_v$$

Equation 15.

The numerical values of E_J have been reported to range from 4 to 92 kJ/mol, overlapping with the ΔH_v range for water and various organic substances relevant to pervaporation separation.[2] This suggests that E_P may take on negative values. While E_D is generally positive, ΔH_S is typically negative for exothermic sorption processes. When the negative ΔH_S outweighs the positive E_D, E_P becomes negative, indicating a decrease in the membrane permeability coefficient with rising temperature. This occurs even though the permeation flux usually increases with temperature, driven by an enhanced driving force.

2.1.5. Configuration of Membranes.

An effective strategy to increase the efficiency of pervaporation involves the introduction of asymmetricity into the membrane structure, to reduce the effective thickness of the membrane, and the use of hollow fiber membranes.

2.1.5.1. Asymmetric Membranes.

Asymmetric membranes are usually fabricated from a single polymer solution using the phase inversion process. This method involves converting a homogeneous polymer solution into a two-phase system, where a polymer-rich solid phase creates the rigid membrane structure, and a polymer-poor liquid phase forms the voids. To produce such membranes, the polymer must first be dissolved in an appropriate solvent. The resulting homogeneous solution is then shaped into a film to achieve the desired geometry (*i.e.* flat, tubular, or hollow fiber). The polymer solution undergoes a controlled process to induce polymer precipitation, followed by a suitable drying step. During the precipitation step, various techniques have been developed to facilitate the phase inversion, like:

1. Precipitation from the vapor phase;
2. Precipitation through controlled evaporation;
3. Precipitation *via* immersion in a nonsolvent;
4. Thermal precipitation;
5. Combinations of these methods.

The separation performance of an asymmetric membrane is generally attributed to its top skin layer. As a result, efforts in the development of asymmetric membranes often prioritize minimizing the thickness of this layer. However, reducing the skin layer thickness increases the likelihood of defects (such as large pores) forming, which can compromise selectivity.[2]

Quantitatively, the selectivity of these membranes can be evaluated using the resistance model, which establishes a correlation between membrane selectivity and the resistance components encountered along the mass transport pathway through the membrane. Additionally, it provides a mean to qualitatively assess these resistances, at least in relative terms. According to this model, a permeating species must traverse the dense skin layer, followed by the substrate's pores and polymer matrix, with the total resistance R_t determined by the following equation (Equation 16):

$$R_t = R_1 + \frac{(R_2 R_3)}{(R_2 + R_3)}$$

Equation 16.

where R_1 is the resistance of the skin layer and R_2 and R_3 are the resistances of the pores and the polymer matrix, respectively.
Therefore, it has been shown that the selectivity achievable in asymmetric pervaporation membranes is influenced not only by the resistance of the skin layer and the substrate, but also by the relative resistance between the polymer matrix and the pores within the substrate.[2]

2.1.5.2. Composite Membranes.

A composite membrane is composed of a skin layer and a substrate, each made separately and from different materials. The preparation of composite membranes involves two main steps: first, casting the microporous support, and then depositing the selective dense layer (barrier) into its surface. A key advantage of this approach is the ability to use different polymers for the barrier layer and the porous support, enabling the combination of properties not achievable with a single material. Common methods for creating composite membranes include lamination of separately cast layers, direct coating of a polymer solution into a support followed by post-treatment, and *in situ* formation of the barrier layer on a microporous support.[2]

The resistance model approach has also been used to evaluate the pervaporation performance of composite membranes. Because the skin layer and substrate are created separately, the substrate can be characterized independently.

2.1.5.3. Hollow Fiber Membranes.

Another effective strategy to increase the efficiency of pervaporation involves the use of hollow fiber membranes, which are designed similarly to shell-and-tube heat exchangers. Due to this structure, the offer several benefits:

1. A much higher membrane packing density compared to flat membranes;
2. They are self-supporting, unlike flat membranes which require external mechanical support;
3. When operated in shell-fed mode, the hollow fibers themselves act as the vacuum vessel.

However, when the permeate is removed from the fiber bores, the narrow channel for permeate flow often leads to a significant pressure buildup inside the fibers, which is

typically more pronounced than with flat membranes. This pressure increase reduces the driving force for pervaporation. To quantitatively describe the permeate productivity and the pressure distribution of permeate vapor within the hollow fibers, explicit expressions from the combination of the pore flow model and the solution-diffusion model can be obtained:

$$p = \left[\frac{a}{b} - \left(\frac{a}{b} - p_L^2\right)\frac{cosh\left(\sqrt{bc}Z\right)}{cosh\left(\sqrt{bc}L\right)}\right]^{0,5}$$

Equation 17.

$$m_L = \frac{1}{\sqrt{bc}}(a - bp_L^2)tanh\left(\sqrt{bc}L\right)$$

Equation 18.

where *p* is the local pressure of the permeate vapor at a differential distance of Z, measured from the closed end of the fiber, p_L is the applied pressure at the fiber opening, L is the fiber length, and m_L is the permeate productivity of the fiber. a, b and c are three parameters that account for the pressure dependence of permeation flux, the viscosity of the permeate vapor (μ), and the fiber inner (d_i) and outer (d_o) diameter:

$$a = a_o\pi(d_o - d_i)/\ln(d_o/d_i)$$

Equation 19.

$$b = b_o\pi(d_o - d_i)/\ln(d_o/d_i)$$

Equation 20.

$$c = 256RT\mu/(\pi d_i^4)$$

Equation 21.

These equations highlight the importance of identifying an optimal fiber diameter, corresponding to maximum productivity.[2]

2.2 Pervaporation for the Organic Phase.

Pervaporation is well-suited for the purification of liquid blends composed of small molecules, including water and organic solvents.
Solvent dehydration remains the primary focus of both academic and industrial research on pervaporation, as it enables azeotrope separation (*e.g.* alcohol/water) through water affinity and size-selective hydrophilic membranes, without requiring an entrainer. Over the past two decades, pervaporation has also been extensively explored for removing trace of volatile organic compounds (VOCs) from water, and for the separation of organic/organic mixtures with close physicochemical properties (aromatic/aliphatic).[19]

Based on the solution-diffusion mechanism, both the chemical characteristics (*e.g.*, functional group interactions) and structural attributes (*e.g.*, pore dimensions, membrane thickness) of a pervaporation membrane can be customized to optimize sorption-diffusion behaviour, selectivity, and consequently permeability and separation efficiency. Clearly, both the inherent properties of membrane materials and their fabrication methods are crucial in defining the overall separation capability. Furthermore, affinity-driven interactions and molecular sieving effects serve as key parameters for selecting appropriate membrane materials for pervaporation-based separation of specific mixtures.

2.2.1. Polymeric Membranes.

Polymeric materials are among the most extensively researched and commonly utilized membrane types, operating based on the solution-diffusion principle in pervaporation. The mixed components initially dissolve on the membrane's surface, with the adsorption rate influenced by the polymer's affinity for each component. They then diffuse through the membrane, where the diffusion rate is governed by the polymer's free volume and the molecular dimensions of the permeating species.

Affinity is the primary factor in selecting polymers for pervaporation membranes. Based on this parameter, hydrophilic polymers are employed for membranes that favor water over organics, while hydrophobic polymers are used for the opposite selectivity. A wide range of hydrophilic polymers, including poly(vinyl alcohol) (PVA), polyelectrolyte complex (PEC), polyamide (PA), and polyimide (PI), are available for pervaporation membranes.
By contrast, the variety of hydrophobic membranes is significantly smaller due to the limited availability of suitable hydrophobic materials. Only a few, such as polydimethylsiloxane (PDMS) and poly(ether-block-amide) (PEBA), can be structured into effective pore configurations for molecular separation.
Therefore, separating organic-organic solvent mixtures is even more challenging. This is due, firstly, to the similar structural and chemical properties of the organic components and, secondly, to the increased likelihood of polymeric membranes swelling in organic solvents. However, several polymeric materials, such as polyimide (PI), chitosan (CS), and

polyethylene glycol (PEG), have been documented for the separation of aromatic/aliphatic and polar/non-polar mixtures, as well as for gasoline desulfurization.[20]

2.2.1.1. Dehydration of Organics.

Hydrophilic polymer membranes are effective for the selective extraction of water from organic solvents. Their hydrophilic nature promotes the preferential absorption of water over the organic solvent, and water molecules diffuse more rapidly due to their smaller molecular size compared to the organic solvent. Polyvinyl alcohol (PVA) was among the first membrane materials used commercially for pervaporation and remains the standard hydrophilic polymer for solvent dehydration.[21] Its hydrophilic nature is attributed to the presence of pendant hydroxyl groups, while its semicrystalline structure arises from the two carbon atoms in its backbone. Although PVA membranes offer excellent selectivity for water, they typically exhibit a relatively low permeation flux (generally below 300 g/m^{2}h). additionally, PVA's water solubility leads to membrane swelling in aqueous environments, which can be minimized through various crosslinking methods, but with a loss of permeation flux.[22]

As a result, in recent years, research on pervaporation membranes for solvent dehydration has focused on developing new materials with enhanced chemical and thermal stability, capable of handling aggressive solvents at higher operating temperatures. In this context, polymers featuring stiff and rigid chains have emerged as promising candidates for such applications. Moreover, the glassy nature of these polymers can enhance the membranes' diffusivity selectivity during the dehydration process, as water molecules are smaller than most organic molecules.[23]

Within this framework, amorphous perfluoropolymers represent a category of high free-volume materials that offer excellent thermal and chemical stability, making them well-suited for handling aggressive solvents. Membranes based on these polymers have been investigated by various research groups for the dehydration of solvents such as butanol, isopropanol, ethanol, N,N-dimethylformamide (DMF), N,N-dimethylsulfoxide (DMSO), N,N-dimethylacetamide (DMAc), and hydrogen peroxide (H_2O_2).[24–29] Despite their inherent hydrophobic nature, a size exclusion mechanism has been identified as the primary separation process in dehydration applications using these amorphous perfluoropolymer membranes.[25]

On the other hand, aromatic polymers like polybenzoxazole (PBO), polybenzoxazinone (PBOZ), and polybenzimidazole (PBI) have also been identified as highly promising materials for solvent dehydration due to their outstanding chemical and thermal stability. Both PBO and PBOZ membranes are produced through a thermal rearrangement process from their corresponding precursors and have proven effective in the dehydration of alcohols.[30,31]

To enhance the efficiency of pervaporation membranes, sulfonation and crosslinking are widely used as modification techniques. Sulfonation is a two-step process that involves initially immersing the polymeric membrane in sulfuric acid, followed by heat treatment at elevated temperatures. While it is commonly believed that the improved

performance of sulfonated membranes is mainly due to increased hydrophilicity and the stronger affinity of water to the membrane – thanks to the introduction of sulfonic groups – some studies suggest that diffusion selectivity plays a more critical role in separation efficiency, primarily due to the significant size difference between water and organic solvent.[32,33]

On the other hand, crosslinking strengthens the polymer network by tightening the polymer chains, which helps reduce membrane swelling and enhances dehydration performance. Depending on the reaction mechanism, crosslinking can be achieved through either thermal or chemical methods. Although crosslinking generally improves the separation factor, it can also reduce the permeation flux because of the excessive densification of the selective layer.[13]

2.2.1.2. Recovery of Organics.

Pervaporation is a viable method for extracting organic substances from water-based solutions, when their concentration is relatively low. Unlike dehydration, the pervaporation process for removing trace amounts of organic compounds necessitates the selective permeation of larger organic molecules through the membrane. Therefore, the hydrophobic membrane (also referred to as organophilic) can achieve selective permeation of organic compounds over water solely through sorption selectivity rather than diffusion selectivity, as organic molecules have lower diffusivity compared to water due to their larger molecular size. In general, the more similar the solubility parameter of a substance is to that of the membrane material, the greater the membrane's attraction to that substance. The solubility parameters of small molecules can be determined by considering the effects of hydrogen bonding, polarity, and dispersive forces.

Polydimethylsiloxane (PDMS), commonly known as silicone rubber, is the most extensively researched hydrophobic material and it is often considered the benchmark for this application. PDMS membranes demonstrate outstanding separation performance for recovering organic compounds from aqueous solutions, along with additional benefits such as high hydrophobicity, ease of processing, and stability.

In the development of pervaporation membranes, two main types of commercial PDMS precursors are frequently utilized, distinguished by their terminal groups. The first type is hydroxyl-terminated PDMS, which is typically crosslinked with tetraethoxysilane (TEOS) through a condensation reaction, resulting in a three-dimensional network structure. The second type is a vinyl-terminated PDMS, which undergoes an addition reaction with a hydrosilyl-containing crosslinker, forming a linear architecture. Research has also shown that four-harmed quaternary-siloxy groups can generate free volume and hydrogen bonding sites, enhancing the sorption and diffusion of phenol molecules within the crosslinked hydroxyl-PDMS membrane.[34]

Beyond PDMS, other derivatives such as polyoctymethylsiloxane (POMS) and polymethylphenylsiloxane (PMPS) have also been explored for their potential in hydrophobic pervaporation membranes.[35]

PEBA, a block copolymer composed of polyamide (PA) and polyether (PE), represents another extensive class of polymers used in hydrophobic pervaporation and gas separation membranes. The rigid PA segment ensured mechanical durability, while the PE segment enhances affinity for organic compounds and polar gases. Due to these properties, PEBA membranes have demonstrated significantly higher phenol/water separation performance than PDMS membrane, making them well-suited for the selective permeation of large organic molecules, such as phenol.[36]

Other hydrophobic polymers with high free-volume fractions have been investigated as highly permeable membrane materials for the pervaporation-based extraction of organic compounds from aqueous solutions. Earlier research focused on poly(1-trimethylsilyl-1-propyne) (PTMSP), where the bulky trimethylsilyl side groups generate abundant free volume, facilitating the selective permeation of molecules.[37] More recently, polymers of intrinsic microporosity (PIMs) have emerged as promising membrane materials for alcohol recovery from water. In Section 3. PIMs will be explored in more detail.

2.2.1.3. Organic/Organic Separation.

The separation of organic/organic mixtures is critically important in the chemical industry, and it is known to be an energy-intensive process. Polymeric pervaporation membranes have been explored for the separation of three primary types of organic mixtures:

1. Aromatic/aliphatic combinations (such as benzene/cyclohexane and toluene/n-heptane);
2. Polar/non-polar mixtures (like methanol/methyl tert-butyl ether);
3. Gasoline desulfurization (model mixtures such as thiophene/n-heptane).

Compared to organic/water mixtures, organic/organic separations present greater challenges for polymeric membranes. This is mainly due to the significant swelling of polymers in pure organic solutions, which compromises the membrane's size-selectivity and structural stability. Additionally, in certain mixtures – such as aromatic/aliphatic systems – the organic components often share similar physicochemical properties, leading to minimal differences in solubility and diffusivity within the membrane, further complicating the separation process. However, numerous polymer-based materials have been documented for the separation of aromatic/aliphatic and polar/non-polar mixtures, as well for gasoline desulfurization.

Polyimide (PI), featuring a benzene ring and a polar imide group on its chain, is a widely used membrane material for separating aromatic/aliphatic mixtures. The benzene ring can engage in π-π interactions with aromatic compounds, while the polar imide group can also interact with the π electron cloud of these compounds. As a result, PI membranes can separate aromatic/aliphatic mixtures based on affinity.[38]
The incorporation of groups with a stronger affinity for benzene ring onto the PI chain could enhance the membrane's selectivity for aromatics. Indeed, the inclusion of siloxane can significantly boost the permeation flux of PI membranes, with the increase

in separation factor attributed to changes in the solubility coefficient. Additionally, introducing fluorine groups into PI can effectively improve its solubility in organic solvents while simultaneously increasing the membrane's free volume.[39] Also crosslinking can significantly enhance the swelling resistance of PI membranes in organic solvents.

Chitosan (CS) is a naturally degradable biological polysaccharide with amino and epoxy groups that give it good hydrophilicity, chemical stability, and membrane-forming capabilities, making it an excellent membrane material.[40] Due to its hydrophilic nature, CS membranes are commonly used in the separation of polar/non-polar mixtures.[41] However, pure CS membranes face several challenges during pervaporation, including swelling in water, dissolution in acids, and low mechanical strength, which restrict their practical use. Additionally, the separation performance of pure CS membranes is limited. Therefore, modifying CS membranes is crucial, with techniques like crosslinking, grafting, blending, and composite membrane creation.[40]

Block copolymers are significant materials for separating organic/organic solvent mixtures through pervaporation, such as poly(ether-block-amide) (PEBA)[42], polyurethane (PU)[43], and others. The unique feature of these polymers is the presence of hard and soft segments: the hard segment enhances the membrane's rigidity and provides better swelling resistance in organic solvents, while the soft segment has a higher affinity for organic compounds. By carefully designing the ratio of soft and hard segments, a balance between separation efficiency and membrane stability can be achieved.[44]

2.3 PIMs for Pervaporation of Organic Solvents.

Polymers of Intrinsic Microporosity (PIMs) represent a novel class of advanced amorphous organic polymer materials characterized by a microporous structure formed by a continuous network of interconnected intermolecular voids. First developed by McKeown and Budd in 2002[45], PIMs distinguish themselves from conventional polymers by their intrinsic porosity, high solubility, large surface area, well-defined pore architectures, and the possibility of controlled post-modification through the designability of synthesis monomers.

These unique properties have positioned PIMs as highly promising materials, particularly in membrane-based separation technologies, attracting significant research interest over the past decade. Initially explored for heterogeneous catalysis, their applications have since expanded to hydrogen fuel storage, gas adsorption, pollutant removal, electrochemistry, fuel cells, and luminescence. However, among these diverse applications, membrane-based separation remains the primary focus, as the inherent characteristics of PIMs make them particularly well-suited for addressing complex separation challenges.

2.3.1. Principles of PIMs.

PIMs possesses multiple characteristics that define their specific applications and impact separation efficiency in membrane technology. The key to developing PIM-based membranes with outstanding separation performance and stability lies in the strategic design of PIMs, leveraging diverse property combinations.

2.3.1.1. Synthesis and Structure of PIMs.

PIMs typically consist of two main components: a structural unit featuring cavities and a contorted site, and a linking group that connects these structural unites while preventing the aggregation of neighbouring components. The fused-ring configuration of the linkage monomer and the spiro-centre unit contribute to rigidity and inherently twisted backbone chains, respectively, which together create an intrinsically microporous structure.

Various type of step-growth polymerization can be employed to synthesize PIM materials, primarily involving the formation of dibenzodioxin, TB (Tröger's base), and imide linkages. It has become evident that the dioxane formation reaction serves as a versatile approach for synthesizing PIMs from suitable hydroxylated aromatic monomers and fluorinated (or chlorinated) aromatic monomers. This reaction is among the few capable of simultaneously forming two covalent bonds with sufficient efficiency to generate a fused ring linking groups and produce ladder polymers with a high average molecular mass. Consequently, it is essential to incorporate a highly rigid and nonlinear linking group to ensure inefficient packing and prevent structural relaxation.

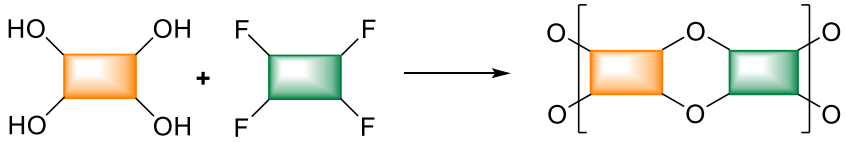

Figure 10.. Polymerization reaction.

To gain a clearer insight into this step-growth polymerization process, the synthesis of PIM-1 is supplied (Figure 11). This reaction involves the dioxane-forming reaction is an irreversible base-catalysed aromatic nucleophilic substitution (S_NAr) reaction, employing 5,5',6,6'-tetrahydroxy-3,3,3',3'-tetramethyl-1,1'-spirobisindane (TTSBI) and 2,3,5,6-tetrafluoroterephthalonitrile (TFTPN). This dual S_NAr reaction generally proceeds efficiently, particularly when the monomer incorporates electron-withdrawing groups (such as the nitrile functionalities, -CN in TFTPN), which assist in activating the dibenzodioxin linkages throughout the step-growth polymerization process. Additionally, the nitrile moiety enables a variety of post-synthetic modifications, which can enhance polymer cohesion and decrease microporosity. Moreover, the four methyl groups present in spirobisindane (SBI) units contribute to increasing interchain spacing in PIM-1, thereby ensuring its solubility in organic solvents. Furthermore, these methyl groups occupy the benzylic positions of PIMs, significantly improving oxidation resistance by preventing easy hydrogen abstraction via free radicals.[46]

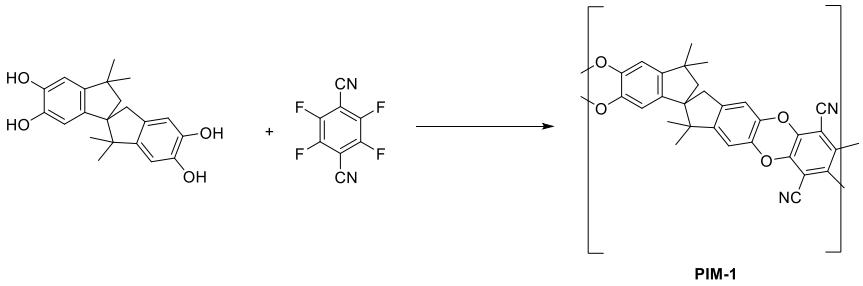

Figure 11. PIM-1 synthesis.

The key factors affecting the synthesis conditions of PIMs include monomer purity, reaction reagents, monomer concentration, temperature, catalyst, and atmosphere (typically inert gases). These elements play a crucial role in determining the reaction rate, product yield, and polymer molecular weight. The conventional approach, known as the low-temperature method, consists of dissolving equimolar amounts of the two monomers in ultra-dry dimethylformamide (DMF) and reacting them with an excess of dry potassium carbonate (K_2CO_3) at a temperature of 50-60°C for a duration of 24 to 72 hours.

2.3.1.2. Key Features of PIMs.

A diverse range of PIM materials for membrane applications can be developed by designing highly rigid monomers and incorporating nonlinear linking groups. Beyond their intrinsic microporosity, which ensures high permeability, PIM materials intended for membrane separation should also exhibit good solubility for easy solution processing, as well as flexibility in design and modification to enable precise separations.

2.3.1.2.1. Solubility.

In membrane processing, a key advantage of polymers is their ability to be easily processed into membranes using the straightforward solution-casting method. Consequently, the solubility of PIMs plays a crucial role in determining their suitability for membrane applications, making solution-processable PIM materials highly desirable for large-scale membrane fabrication. Additionally, the solubility of PIMs enables characterization through solution-based techniques such as gel permeation chromatography (GPC), which provides valuable insights into polymer molecular weight and helps assess its film-forming ability and mechanical properties.

Furthermore, whether a polymer dissolves in each solvent is significant, as the choice of solvent can greatly impact the final membrane's performance. Solubility can be evaluated using the solubility parameter (δ), which consists of three key components with varying degrees of influence: hydrogen bonding contribution (δ_h), dispersion force contribution (δ_d), and polar force contribution (δ_p). The overall δ value is determined by the sum of the squares of these three components.[46]

Polymer solubility can be enhanced by designing monomers with rigid and contorted chain structures, which hinder efficient packing and create sufficient intrinsic microporosity.[47] Additionally, incorporating specific substituent groups – such as alkyl groups, naphthalene units, flexible side chains, and trifluoromethyl (-CF_3) groups – can further improve solubility. In particular, the -CF_3 group, known for its "fluorine effect", enhances polymer solubility by increasing chain rigidity and reducing interchain interactions, while also improving membrane permselectivity, especially in gas separation applications.

Moreover, most PIMs with a non-network configuration are generally soluble in organic solvents. For example, PIM-1 exhibits excellent solution processability in chloroform ($CHCl_3$), tetrahydrofuran (THF), and other solvents.

2.3.1.2.2. Intrinsic Microporosity.

Intrinsic microporosity (IM) is defined as "a continuous network of interconnected intermolecular voids, which forms as a direct consequence of the shape and rigidity of the component macromolecule".[48] In other words, the unique structure of these macromolecules prevents efficient packing, leading to the formation of permanent voids within the material. This characteristic is often referred to as high free volume, highlighting the significant amount of unoccupied space within the polymer matrix. When discussing PIMs, a deeper understanding of IM requires an examination of fractional free volume (FFV) and the relevant characterization methods.

On the one hand, the free volume in a polymer sample refers to the space not occupied by the polymer molecules, while the fractional free volume (FFV) is commonly used to quantify the percentage of this unoccupied space.[49] FFV is a key parameter to describe the relative "empty space" in a polymer membrane, which arises due to inefficient packing of the polymer chains. To calculate FFV, one of the most widely used methods is Bondi's group contribution approach. It is generally assumed that disrupting the

packing of the chains increases the FFV, which in turn can enhance gas permeability due to the larger available space for gas molecules to diffuse.
On the other hand, the specific surface area (S_{BET}) measurement is frequently employed to analyse the microporous structure of PIMs.[50] However, the use of S_{BET} to characterize the porosity of polymeric materials remains a topic of debate, as it may not always accurately reflect the true porous nature of these polymers.

2.3.1.3. Physical Aging and Strategies for Mitigation.

PIMs exhibit distinct, rigid, and twisted macromolecular backbones that lead to poor molecular packing, resulting in an interconnected and irregular microporous structure. However, despite these unique characteristics, PIMs encounter significant issues related to physical aging and plasticization – two interrelated and widely recognized challenges that impact the industrial applicability of polymer membranes, particularly high-permeability PIM-based membranes for gas separation. Plasticization refers to the expansion of polymer membranes when exposed to condensable gases (such as CO_2), leading to increased chain mobility and accelerated diffusion of gas molecules through the membrane. While this increases permeability, it simultaneously lowers the membrane's separation selectivity.[51,52]

Unlike plasticization, which is caused by the external adsorption of condensable gases and the increased gas concentration within the membrane, physical aging involves reversible changes in the material over time, independent of external factors.[53,54] This distinction highlights the contrasting mechanisms driving chain motion in each process. Moreover, the rate of physical aging is influenced by factors such as temperature, surrounding conditions, gas properties, and polymer structure. Specifically, even when in a kinetically restricted glassy state below the glass transition temperature, PIMs retain excess free volume and inevitably experience localized segmental motions. These movements gradually reduce the interstitial space over time as the material shifts toward thermodynamic equilibrium. Over time, physical aging slows as the available free volume diminishes, reducing polymer chain mobility and segmental motion. As a result, the packing density of PIM-based membranes increases, causing a reduction in the initial free volume. This leads to a decline in gas permeability while simultaneously enhancing separation selectivity. To counteract this unwanted effect and ensure stable, long-term membrane performance in industrial applications, it is crucial to minimize physical aging as much as possible.[55–57]
To address the physical aging of PIM-based membranes, several strategies have been investigated, including the development of novel PIMs with rigid structural units or unique functional groups, treatments such as methanol exposure[58], thermal processing[59] (*e.g.* crosslinking or rearrangement), and post-modification[60]. The key to minimizing physical aging lies in the polymer's intrinsic molecular structure, where the incorporation of rigid units enhances chain stiffness, restricting segmental motions and improving membrane stability over time.

2.3.2. Overview of Fabrication Strategies for PIMs-Based Membranes.

The structural characteristics and intrinsic properties of PIMs play a crucial role in defining both their application scope and the separation efficiency of PIMs-based membranes. From a rational design perspective, an ideal PIMs-based membrane should exhibit high separation performance, prolonged operational stability, and adequate component compatibility. At the same time, its fabrication process should be straightforward, scalable, and suitable for large-scale production. Initially, PIM-1, the most widely recognized PIM, along with other extensively synthesized PIMs materials, was primarily utilized as a polymer matrix for separation membranes, particularly in gas separation applications. Currently, the main fabrication techniques for PIMs-based membranes encompass solution casting, blending, and surface modification approaches such as coating, dip-coating, and self-assembly. Additionally, methods including post-modification, interfacial polymerization, and phase inversion are also commonly employed to enhance membrane properties and performance.[46]

One of the most common methods for fabricating PIMs-based membranes is solution casting, a simple yet effective technique. This process begins with the preparation of a polymer solution, where PIMs are dissolved in an organic solvent, such as chloroform ($CHCl_3$), at a controlled concentration. To ensure complete dissolution, the mixture undergoes continuous stirring for several hours, sometimes supplemented with ultrasonic treatment. Once homogeneous, the solution is filtered through a polytetrafluoroethylene (PTFE) membrane to remove any undissolved polymer residues before being cast into a flat-bottomed Petri dish. The solvent then evaporates gradually under stable environmental conditions, leading to membrane formation over a period ranging from several hours to a few days. After the initial film is formed, the membrane is further dried in a vacuum oven at elevated temperatures for up to 48 hours to remove residual solvents and improve structural integrity. In some cases, an additional methanol treatment step is performed prior to vacuum drying to eliminate trapped solvents and impurities, ensuring the production of dense, isotropic membranes with optimized properties.[46]

Beyond their fabrication, PIMs-based membranes are widely used to their ease of processing, structural flexibility, and relatively low production costs. However, like conventional polymeric membranes, they face challenges such as performance degradation over time due to aging and the inherent trade-off between permeability and selectivity. To overcome these limitations, researchers have explored various strategies, including the design of novel monomers, post-synthesis modifications, and copolymerization techniques. A particularly promising approach involves blending PIMs with nanomaterials to create mixed-matrix membranes (MMMs), which combine the high separation efficiency of nanofillers with the processability of PIMs. This strategy not only enhances membrane performance but also mitigates the aging effects that typically compromise long-term stability. However, a key challenge remains in

identifying suitable filler materials and ensuring their compatibility with the polymer matrix. Various fillers have been investigated, including conventional polymers, non-porous nanoparticles (*e.g.*, polyhedral oligomeric silsesquioxane, POSS), carbon-based materials (*e.g.*, graphene oxide, GO; carbon nanotubes, CNTs; graphitic carbon nitride, g-C_3N_4), as well as metal-organic frameworks (MOFs), microporous organic polymers (MOPs), ionic liquids, and metal salt-based nanocomposites.[46]

Given the challenges associated with self-standing PIMs membranes, an alternative approach involves fabricating asymmetric composite membranes or depositing ultrathin selective layers onto porous supports, offering improved separation efficiency and long-term performance. Thin-film composite (TFC) membranes, for instance, are produced by coating a selective layer onto a porous substrate, ensuring high permeability while maintaining structural integrity. When these TFC membranes are further enhanced with nanofillers, they are referred to as thin-film nanocomposite (TFN) membranes, which exhibit superior separation characteristics. The fabrication of these advanced composite structures must be carefully optimized to ensure that the deposition process does not alter the intrinsic properties of the pristine membrane. With their ability to achieve high-performance gas separation and enhanced stability, TFN membranes represent a promising direction for industrial applications.[46]

2.3.3. PIMs-Based Membranes for Pervaporation: Applications and Prospects.

Due to their microporous structure, customizable monomer design, and ease of membrane fabrication, PIMs-based membranes are gaining attention for applications in both gas and liquid separation. These membranes have been developed as strong contenders, offering unique properties for a range of membrane-based separation processes. Currently, most PIMs-based membranes are primarily utilized in gas separation, with growing use in other separation techniques such as nanofiltration (NF), organic solvent nanofiltration (OSN), pervaporation (PV), and fuel cells.

Building on the growing interest in PIMs-based membranes for various separation applications, pervaporation (PV) stands out as a key area where these materials show significant promise, particularly for dehydration processes. As such, PIMs materials must meet specific performance standards to be effective for PV, with PIM-1 being among the first to be applied for the dehydration of aliphatic alcohols such as methanol (CH_3OH), ethanol (C_2H_5OH), isopropanol (C_3H_7OH), and butanol (n-C_4H_9OH)[61]. Similarly, various studies have explored the selective removal of butanol from aqueous solutions[62], the separation of methanol-dimethyl carbonate[63], the purification of ethylene glycol (EG)[64], and the elimination of volatile organic compounds from water using PIM-1 membranes in pervaporation processes. Due to the inherently hydrophobic properties of most PIMs materials, their application in pervaporation is primarily focused on removing organic substances from aqueous mixtures.

To further expand their applicability in pervaporation, strategies such as post-synthetic modifications and polymer blending are being explored to optimize membrane performance, making PIMs-based membranes even more effective for precise and energy-efficient separation processes. Using PIM-1 as a representative case, numerous post-synthetic modifications have been carried out on its -CN groups due to their facile and rapid reactivity. For instance, Guiver and Co[46]. successfully transformed the nitrile functionalities into carboxylic acid, with the extent of hydrolysis being largely dictated by reaction temperature and duration. The resulting PIM-1-COOH exhibits solubility in high-boiling polar solvents such as DMSO, DMAc, and DMF, whereas it remains poorly soluble in THF and $CHCl_3$. Moreover, extensive research has been conducted on alternative post-synthetic modifications of PIM-1, enabling the conversion of its nitrile groups into various functional moieties, including amidoxime[65], methyl tetrazole[66], thioamide[67], and ketone[68].

These advancements highlight the versatility of PIMs-based membranes and their potential for further optimization in separation technologies. By tailoring their chemical structure and enhancing their functional properties, researchers can continue to expand their applicability beyond pervaporation, paving the way for more efficient and selective membrane-based processes in industrial and engineering applications.

2.4 Separation of C3–C5 alcohols by Pervaporation

2.4.1 Separation of Propanol, Butanol, and Pentanol by Pervaporation

2.4.1.1 Membrane Materials for Alcohol Separation

PV is a promising technology for separating longer-chain alcohols like propanol, butanol, and pentanol due to its low energy consumption and high efficiency in treating dilute mixtures, such as those resulting from fermentation processes. Recent research highlights the effectiveness of PV with composite membranes based on hydrophobic polymers such as PDMS and innovative materials like PIMs.

PDMS-based membranes are widely used for the pervaporation of alcohols due to their high permeability to organic compounds. PDMS is a rubbery silicone polymer with high free volume, which facilitates the diffusion of organic molecules such as butanol while excluding a significant fraction of water. Despite these advantages, PDMS membranes often face a trade-off between flux and selectivity. For instance, a composite PDMS membrane demonstrated a butanol/water separation factor of ~32 with total flux ~0.25 $kg \cdot m^{-2} \cdot h^{-1}$ for a 1 wt% n-butanol feed, showcasing the material's efficiency in alcohol recovery.

To improve performance, PIM-1 has emerged as a promising alternative. PIM-1 is a rigid, contorted polymer with high free volume, which yields very high permeability for organics. PIM-1 membranes demonstrate strong alcohol flux performance but moderate selectivity when fresh. For example, a fresh PIM-1 membrane had a butanol/water selectivity of ~2.8 at a 2 wt% feed, which increased to 5.1 upon physical aging over 370 days (Figure 12). This improvement was attributed to polymer chain relaxation and pore contraction, which reduce water permeability more significantly than butanol's, improving selectivity[69].

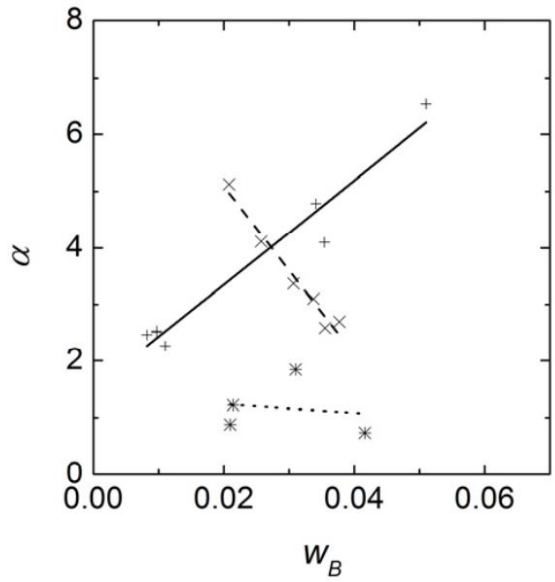

Figure 12.: Selectivity of 1-butanol over water at 50 °C in PIM-1 membranes as function of the weight fraction of butanol in the feed. Symbols: (–+–) fresh membrane; (----) aged membrane (12 months); (····X····) further aged membrane (additional 6 months), immediately after subsequent MeOH soaking (total 18 months old) from Figure 4[69].

Hybrid membranes combine PDMS and PIM-1 to leverage the complementary properties of both materials. These membranes combine the flexibility and film-forming ability of PDMS with the intrinsic microporosity of PIM-1, enabling both improved flux and higher selectivity. Zhang et al. (2019) reported a PIM-1/PDMS hybrid membrane achieving a remarkable permeate flux of 1425 $g \cdot m^{-2} \cdot h^{-1}$ and a separation factor of 30.7 for n-butanol at 1.0 wt% concentration in water[70].

Other hybrid strategies include filling PDMS with zeolites or carbon materials to enhance performance. For example, Silicalite-1, a hydrophobic zeolite, embedded in PDMS improved butanol selectivity ($\alpha \approx 32$) but slightly reduced flux. Similarly, PDMS membranes with 10% carbon nanotubes (CNTs) achieved $\alpha = 32.9$ and total flux ~0.24 $kg \cdot m^{-2} \cdot h^{-1}$ at 80 °C, demonstrating an effective balance between selectivity and productivity.

2.4.1.2. Performance Comparison for Propanol, Butanol, and Pentanol

The separation performance of these alcohols varies due to their differences in volatility, polarity, and molecular size:

Propanol: Fully miscible with water, propanol requires membranes with high selectivity. PDMS membranes often exhibit separation factors in the range of 5–15, with fluxes exceeding water under certain conditions due to propanol's relatively higher vapor pressure. However, hybrid membranes with zeolite or CNT fillers have demonstrated improved selectivity while maintaining satisfactory flux.

Butanol: Butanol has limited solubility in water and displays superior sorption selectivity in hydrophobic membranes. PDMS separation factors for butanol typically range from

~20 to over 50, achieving permeate purity exceeding 99% by weight for dilute butanol solutions[71]. Hybrid PIM-1/PDMS membranes achieved flux as high as 1.4 $kg \cdot m^{-2} \cdot h^{-1}$ with a separation factor of 30.7, a remarkable improvement over pure PDMS membranes.

Pentanol: Exhibiting lower volatility and diffusivity, pentanol requires higher operating temperatures to achieve reasonable fluxes. Due to its hydrophobic nature, pentanol achieves higher selectivity in PDMS-based membranes compared to shorter alcohols. PIM-1/PDMS hybrid membranes have shown improved pentanol separation efficiency at elevated temperatures, achieving separation factors above 30 with total flux reaching ~0.24 $kg \cdot m^{-2} \cdot h^{-1}$ at 80°C[72].

2.4.1.3 Operating Conditions and Performance Optimization

Pervaporation performance is strongly influenced by:

Feed Concentration: Increasing the alcohol concentration generally enhances total flux but may reduce separation selectivity. For example, increasing n-butanol concentration from 1 wt% to 5 wt% in feed at 60 °C raised total flux from 1.43 to 3.43 $kg \cdot m^{-2} \cdot h^{-1}$ in a PIM-1/PDMS membrane.

Temperature: Higher feed temperatures improve permeation rates by increasing vapor pressure and membrane chain mobility. For instance, raising butanol feed temperature from 30°C to 60°C increased flux by over 100% in PDMS–PIM-1 membranes[70].

Permeate Pressure: Lowering the permeate pressure increases the driving force for mass transfer. Maintaining a vacuum (e.g., 5–10 mbar) ensures optimal performance, particularly for volatile alcohols like propanol.

2.4.2 Separation of Hexanol by Pervaporation

Recent advancements in membrane technology have introduced ionic liquid (IL)-derived organosilica membranes as a highly effective solution for separating hexanol from aqueous mixtures. These membranes combine ionic liquid moieties with silsesquioxane frameworks, providing both chemical stability and selective sorption properties.

The SipmimTf2N membrane, derived from an imidazolium-based IL, demonstrated excellent performance in hexanol separation. This membrane achieved a 1-hexanol/n-hexane separation factor of 2.3 with a permeance ratio of 790. At 293 K, this membrane attained a molar flux of 9.9×10^{-5} mol m^{-2} s^{-1} for 1-hexanol, achieving stable and efficient separation in binary PV tests[72].

The SipmimTf2N membrane exhibited excellent resistance to swelling, a common issue in alcohol pervaporation, and maintained performance even under varied operating conditions. Its selectivity improved by leveraging Hansen solubility parameters (HSPs) to enhance affinity for hexanol while minimizing water co-permeation.

2.5 Conclusions and Perspectives on Alcohol Separation by Pervaporation

The separation of longer-chain alcohols, such as propanol, butanol, pentanol, and hexanol, by pervaporation has shown promising results with significant advancements in membrane technology. The combination of tailored membrane materials, optimized operating conditions, and innovative hybrid designs has enabled substantial improvements in both separation selectivity and permeate flux.

2.5.1. Conclusions

2.5.1.1 Membrane Performance and Material Selection:

PDMS membranes are widely recognized for their high permeability and effective separation of hydrophobic alcohols like butanol and pentanol. However, their selectivity remains moderate when handling dilute solutions or highly volatile alcohols such as propanol.

PIM-1 membranes have emerged as a powerful alternative due to their high free volume and intrinsic microporosity, which enable enhanced alcohol permeation. Nevertheless, PIM-1 membranes initially struggle with selectivity, which improves significantly with aging.

Hybrid membranes, such as PIM-1/PDMS composites, combine the flexibility and processability of PDMS with the superior molecular sieving properties of PIM-1. These designs have delivered some of the most efficient results, with flux exceeding 1.4 kg·m^−2·h^−1 and separation factors above 30.

Advanced fillers like **zeolites** and **carbon nanotubes (CNTs)** further enhance PDMS performance by improving selectivity without compromising permeability. Such hybrid solutions represent a major step forward in achieving optimal membrane performance for long-chain alcohol separation.

2.5.1.3 Operating Conditions:

Increasing the **feed concentration** generally enhances total flux but can reduce separation selectivity. Multi-stage pervaporation systems or recycle strategies may be effective solutions to balance these effects.

Temperature control plays a pivotal role in improving flux by enhancing vapor pressure and diffusion rates. While higher temperatures typically improve flux, excessive heat may reduce selectivity in some membranes.

Permeate pressure control (typically achieved by vacuum conditions) is crucial for maximizing the driving force in the separation process. Optimizing this condition is especially important for volatile alcohols such as propanol and butanol.

2.5.1.3.1-Hexanol Separation:

1-Hexanol presents unique challenges due to its larger molecular size, lower volatility, and strong affinity for hydrophobic membranes. Recent advancements with **ionic liquid-derived organosilica membranes** have shown excellent performance, particularly in overcoming swelling issues that commonly affect PDMS membranes.

The **SipmimTf2N membrane**, developed from an imidazolium-based ionic liquid, achieved remarkable separation performance for hexanol with a separation factor of 2.3 for 1-hexanol/n-hexane mixtures and a molar flux of 9.9×10^{-5} mol m^{-2} s^{-1} at 293 K, maintaining stable performance across a range of operating conditions[72].

2.5.2. Perspectives

2.5.2.1 Material Innovation:

Future research should explore new hybrid membranes that combine PDMS, PIM-1, and ionic liquids with emerging materials such as **metal-organic frameworks (MOFs)** and **graphene oxide**. These materials offer potential for improving both selectivity and stability in alcohol separations.

Further optimization of **nanostructured fillers**, including zeolites, CNTs, and porous carbons, may provide additional performance gains, particularly in enhancing membrane robustness and durability under industrial conditions.

2.5.2.2 Process Optimization:

Multi-stage pervaporation systems should be investigated to enhance recovery from dilute feeds while minimizing energy consumption. This approach should enable the separation of 1-hexanol from complex mixtures, containing water, acetate, ethanol, butanol, caproic acid and butyrate. Improved recycling methods for partially treated streams could further improve process efficiency.

Temperature, pressure, and membrane thickness adjustments must be carefully optimized to ensure maximum separation performance without compromising stability.

2.5.2.3 Long-Term Stability and Industrial Scalability:

Scaling up advanced membrane designs requires careful assessment of membrane stability during prolonged operation. Mechanical robustness, chemical resistance, and resistance to fouling must be ensured for effective long-term deployment.

Developing cost-effective fabrication methods for PIM-based and IL-derived membranes is essential to enable widespread industrial adoption.

By addressing these challenges and leveraging advanced membrane materials, pervaporation technology holds great potential for efficiently separating higher alcohols, including 1-hexanol, from complex mixtures in both biotechnological and chemical process industries.

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3 BIOMASS COMPOSITION, ELEMENTAL ANALYSIS, AND BIOACTIVE COMPOUND CONTENT IN ANOXYGENIC PHOTOTROPHIC PURPLE AND GREEN SULFUR BACTERIA: EXTRACTION METHODS AND CHARACTERIZATION

3.1. Introduction

Anoxygenic phototrophic bacteria are capable of photosynthesis without photolysis of water, which is common for oxygenic phototrophs like cyanobacteria or algae, they use different electron donors instead[1]. In our research, we focus on two ecological groups of anoxygenic phototrophs: purple and green sulfur bacteria (PSB and GSB), which can be found in nature in soil or in sediment at the bottom of water bodies. Both of these groups use hydrogen sulfide as their preferred electron donor for photosynthesis and oxidize it to elemental sulfur, which can be further oxidized to sulfate[2]. Elemental sulfur is stored in globules inside the cells in case of PSB or outside the cells in case of GSB. Another difference is that GSB grow in lower parts of water column in nature and therefore are adapted to higher hydrogen sulfide concentrations, higher reduction potential and lower light intensity[3]. The ability of PSB and GSB to oxidize hydrogen sulfide gives them a biotechnological potential for hydrogen sulfide removal from various matrices, for example biogas[4] or wastewater[5]. A by-product of such technology would be biomass of these bacteria. The goal of this text is to sum up information from the literature about the composition of their biomass, especially elemental composition, content of pigments, vitamins, storage compounds and other bioactive compounds. This knowledge can be useful for finding an application for the biomass, for example as a biofertilizer[6], and thus making desulfurization using anoxygenic phototrophic bacteria more profitable.

3.1.1 Basic components of the biomass

So far, little is known about elemental composition of anoxygenic phototrophic bacteria biomass. In a study[7], electron microprobe X-ray analysis was used for elemental analysis of PSB *Thiopedia rosea* biomass. The results are shown in Table III.

Table III. Elemental analysis of Thiopedia rosea biomass (data from Scherrer & Shull, 1986)

Element	**K_α X-ray intensity [cpm]**
Carbon	8760
Sodium	35
Magnesium	3053
Phosphorus	1740
Sulfur	569
Potassium	350
Manganese	15

Iron	25

In another study, composition of biomass of the PSB *Allochromatium vinosum* was examined via GC-TOF-MS, HPIC and HPLC-MS for several variants of cultivation. Table IV sums up the averages of the most important results. In the study, more detailed information is available about particular compounds from the classes[8].

Table IV. Analysis of Allochromatium vinosum biomass (data from Weissgerber et al., 2014)

Metabolite classes	**Content [nmol/mg protein]**
Amino Acids (non standard)	6.89
Amino Acids (standard)	26.05
Organic Acids	54.88
Fatty Acids	47.90
N-Compounds	36.32
Phosphates	39.08
Polyhydroxy Acids	9.63
Polyols	20.27
Sugars	21.26
MSTs	145.14
Hydrogen sulfide	1.72
O-Acetyl-serine	0.04
Natrium	429.829
Ammonium	227.300
Kalium	1013.489
Chloride	1014.511
Nitrate	14.233
Sulfate	100.463
Phosphate	127.486

From vitamins, only cobalamin is usually added to the culture media[9]. Therefore, it can be assumed that PSB and GSB can produce all the other necessary vitamins on their own. Inositol production was observed in *Allochromatium vinosum*[8]. Studies exist about carotenoid production (see below), which can also be considered vitamins. No information has been found about the production of other vitamins.

3.1.2. Pigments

The main pigments capturing light energy are bacteriochlorophylls. In PSB, we can find bacteriochlorophyll a or b[10], whereas GSB contain bacteriochlorophyll c, d or e[11]. A little amount of bacteriochlorophyll a is also present in the reaction center of GSB[12]. Many species of both PSB and GSB can synthesize bacteriochlorophyll on their own[1013].

Nevertheless, it is recommended to add cyanocobalamin to the medium as a source of tetrapyrrole ring for faster growth[9].

The other major group of pigments present in the PSB and GSB cells are carotenoids. Spirilloxanthin, okenone, rhodopinal and their derivatives are the carotenoids typical for PSB. In GSB, we usually find chlorobactane, β-isorenieratene or isorenieratene and their derivatives. Carotenoids have a major influence on the culture color. PSB with carotenoids of the spirilloxanthin group are usually orange or brownish, with okenone group they are purple red and with rhodopinal group they are purple violet[10]. GSB with chlorobactane group carotenoids are bright green, whereas those with β-isorenieratene, isorenieratene or their derivatives are brown[13]. It is not without interest that these brown GSB grow in lower light intensity than the green ones. Another difference between these subgroups of GSB is that at least some of the green species only contain bacteriochlorophyll c and d, whereas brown ones only contain bacteriochlorophyll e[14].

The pigments can be quantified via UPLC-MS. In a study of several pure PSB cultures, concentration of bacteriochlorophyll a and okenone was measured in the exponential and stationary phase of growth. The highest concentration of both pighments was reached in *Marichromatium purpuratum* culture in exponential phase: 22.3 mM bacteriochlorophyll a and 16.8 mM okenone. However, in all the other cultures, pigment concentrations in stationary phase were higher than in exponential phase[15,16]. Bacteriochlorophyll concentration can also be calculated from absorption spectra, even those of intact cells. This method is especially suitable for measurements directly in the environment[1716].

3.1.3. Inorganic storage compounds

Both PSB and GSB are well-known for accumulating elemental sulfur. PSB form sulfur globules inside their cells whereas GSB produce them outside their cells. Sulfur is not only a byproduct of hydrogen sulfide oxidation, but also an alternative electron donor for photosynthesis when all the hydrogen sulfide present is consumed. Elemental sulfur content in dry culture weight may reach 55 % for PSB and 63 % for GSB[18]. It can be assumed that the sulfur content will be the highest at the moment of the cultivation when hydrogen sulfide concentration drops to zero and then it will decrease as the sulfur will be consumed instead of hydrogen sulfide.

Some species of PSB and GSB have been reported to store polyphosphate globules, namely GSB *Chlorobium limicola*[19] and PSB *Allochromatium vinosum*[20]. Polyphosphate concentration in GSB can reach 2 % of the dry cell mass[18].

3.1.4. Organic storage compounds

Both PSB and GSB are known to accumulate glycogen for carbon and energy storage. Mostly, glycogen content is about 5–10 % of dry cell weight of PSB and up to 35 % in GSB. In some studies, especially with *Chromatium minutissimum*, up to 40 % glycogen was detected. Poly-β-hydroxyalkanoates are another storage compound of anoxygenic

phototrophs. To the best of our knowledge, no GSB produce poly-β-hydroxyalkanoates, but PSB do. The most usual compound of this group in PSB is poly-β-hydroxybutyrate. Its concentration is very variable, the highest concentration was detected in *Thiocystis violacea* growing on acetate: 83 %. Organic storage compounds are produced in highest rate at enough light intensity, presence of organic nutrients such as acetate, and at low concentrations of nitrogen in medium[18].

3.1.5. Other Bioactive Compounds in Phototrophic Bacteria

Beyond their elemental composition, pigments, and storage compounds, anoxygenic phototrophic bacteria produce a diverse array of bioactive molecules with potential applications in biotechnology, medicine, and environmental sustainability. These include antimicrobial compounds, extracellular polysaccharides, and industrially relevant enzymes, many of which contribute to microbial interactions, defense mechanisms, and ecological adaptation. Carotenoids are synthesized by all phototrophic bacteria and have essential roles in photosynthesis as auxiliary pigments. They help protect the photosynthetic system from damage caused by excessive light exposure and play a crucial role in neutralizing reactive oxygen species[21].

3.1.5.1. Antimicrobial Compounds

Certain species of purple sulfur bacteria (PSB) and other anoxygenic phototrophic bacteria synthesize bioactive secondary metabolites with antimicrobial properties. These compounds play an important role in microbial competition and survival by inhibiting the growth of competing microorganisms. Marine purple photosynthetic bacteria, for instance, have demonstrated significant antibiotic activity against *Saccharomyces cerevisiae* and other microbial species, positioning them as promising candidates for novel antimicrobial agents[22].

In addition to these antimicrobial metabolites, certain phototrophic bacteria produce bioactive pigments with defensive properties. Violacein, a deep purple pigment synthesized by various bacterial species, including some phototrophic bacteria, has been studied for its broad-spectrum antibacterial, antiviral, and antifungal effects. Its mechanism of action involves interference with microbial DNA replication and oxidative stress induction, highlighting its potential pharmaceutical and biotechnological applications[23].

These previous literature findings underscore the importance of understanding of biosynthetic pathways of antimicrobial compounds in phototrophic bacteria. Certainly, according to the literature data there is a possibility for large-scale application that would lead to novel antibiotic developments that could serve as biocontrol agriculture agents and support food preservation strategies.

3.5.1.2. Extracellular Polysaccharides

Extracellular polysaccharides are another class of bioactive compounds produced by phototrophic bacteria, playing a role in biofilm formation, environmental adaptability, and stress resistance[24]. Extracellular polysaccharides from *Allochromatium vinosum* and *Chlorobium limicola* contain glucose, mannose, and uronic acids, contributing to their structural integrity and adhesive properties[18]. These biopolymers have diverse industrial applications, including wastewater treatment, heavy metal adsorption, and bioflocculation. Their ability to chelate metals and trap organic matter makes them valuable in environmental detoxification processes[25].

3.5.1.3. Enzymes and Biocatalysts

Anoxygenic phototrophic bacteria also produce a range of enzymes involved in sulfur metabolism, nitrogen fixation, and carbon cycling, making them highly relevant for biotechnological applications[26]. Sulfur oxidases and hydrogenases, in particular, play crucial roles in bioenergy production and environmental bioremediation[27]. These enzymes facilitate the transformation of sulfur compounds and hydrogen metabolism, which can be harnessed for sustainable energy solutions and pollution mitigation.

Additionally, sulfur-rich bacterial biomass derived from these bacteria has several biotechnological applications:

- Sulfur-rich bacterial biomass can enhance soil microbial activity and nutrient availability, serving as biofertilizer[28].
- Carotenoids from purple and green sulfur bacteria hold promise in the food and pharmaceutical industries since they can serve as antioxidant and antimicrobial agents[29].
- The ability of these bacteria to metabolize sulfur compounds and produce extracellular biopolymers makes them useful in wastewater treatment and environmental detoxification, serving as bioremediation[4].

These bioactive compounds illustrate the potential of phototrophic bacteria in sustainable biotechnology. The main issue and the future research should be focused on optimizing their production and application in different industries, including medicine, agriculture and especially environmental management.

3.2. Overview of Biomass Separation Processes

Efficient biomass separation is a crucial step in downstream processing, ensuring the recovery of high-purity biomass while preserving its structural integrity and bioactive compounds. The choice of separation technique greatly influences the overall yield, quality, and economic feasibility of biomass valorization. Various methods have been developed to address the specific challenges associated with biomass recovery, including cell fragility, bioactive compound stability, and process scalability.

This section provides an overview of key biomass separation processes, highlighting their principles, advantages, and limitations. By understanding the distinct features of each technique, researchers and industry professionals can make informed decisions to optimize biomass recovery strategies for various applications.

3.2.1. Common Biomass Separation Techniques

Centrifugation:
Centrifugation is extensively used for separating biomass-rich suspensions due to its high efficiency, rapid processing capabilities, and scalability for industrial applications. This method leverages density differences between microbial biomass and the surrounding liquid medium, allowing for rapid sedimentation of biomass particles and resulting in high biomass recovery rates. By applying centrifugal forces significantly greater than gravitational force, centrifugation efficiently concentrates microbial cells into pellets, substantially simplifying the downstream processing required for biomass valorization.

Centrifugation methods can vary significantly in design and operational parameters, including rotational speed, temperature control, duration, and centrifuge type (e.g., tubular bowl, disc stack, decanter). High-speed centrifugation is typically employed for the rapid separation of small microbial cells or cellular fragments, while moderate-speed centrifugation is suitable for larger cells or cell agglomerates, where lower shear stress is desired to preserve cellular integrity and bioactive compounds.

In applications such as membrane-coupled high-rate algal ponds (M-HRAP) for urban wastewater treatment, centrifugation enhances biomass harvesting efficiency and nutrient recovery. The implementation of centrifugation in conjunction with membrane processes demonstrates increased biomass productivity, nutrient removal rates, and facilitates sustainable reuse of treated effluent for agricultural purposes (Robles et al., 2020). Particularly, centrifugation effectively complements membrane separation processes by rapidly separating biomass from treated effluents, thereby increasing overall operational efficiencies, especially at lower hydraulic retention times (HRT).

However, despite its efficiency, centrifugation typically requires higher energy inputs compared to other separation methods, such as filtration. Thus, when scaling up, careful consideration must be given to optimizing centrifugation conditions to balance the trade-offs between energy consumption, biomass recovery efficiency, and quality preservation.

In conclusion, centrifugation represents a robust method for biomass separation due to its rapid and effective biomass recovery capabilities, making it particularly suitable for applications requiring high-purity biomass. Nonetheless, optimization of operational parameters and integration with complementary low-energy separation techniques are advisable to achieve economic and sustainable viability at an industrial scale[30].

Filtration Systems:
Filtration processes, including microfiltration (MF), ultrafiltration (UF), and membrane-based separations, are pivotal in biomass harvesting. MF is typically employed to separate larger microbial cells, generally greater than 0.1 µm in size, effectively removing suspended solids with minimal energy input and reduced shear stress, thereby

preserving biomass integrity. Hollow-fiber membranes used in MF and UF systems provide high surface-to-volume ratios, optimizing space utilization and enhancing filtration performance, making them particularly suitable for industrial-scale operations. UF is utilized to concentrate smaller particulates, colloids, macromolecules, and dissolved bioactive compounds, ensuring a high-quality biomass product. Membrane-based techniques like UF demonstrate selectivity based on molecular size, allowing for the targeted retention or removal of specific fractions within the biomass suspension. These methods offer several operational advantages, including low energy requirements, controlled separation conditions, and scalability, which are crucial for maintaining biomass integrity and nutrient preservation. The inherent scalability of these membrane processes also enables their direct integration into existing industrial facilities, with low incremental capital and operational costs. Moreover, membrane-based filtration techniques are advantageous due to their lower environmental impact compared to conventional separation methods. They require fewer chemicals and energy inputs, aligning with sustainable development goals. This is particularly relevant in scenarios involving the recovery of valuable bioactive compounds such as vitamins, pigments, and polyphosphates from microbial biomass. However, membrane fouling remains a significant operational challenge. Fouling can lead to a decline in permeate flux and increased operational costs. Effective fouling control strategies, including periodic cleaning cycles, optimization of transmembrane pressures, and strategic aeration or backflushing protocols, are essential for maintaining long-term operational efficiency and reducing downtime[31]. In summary, filtration processes offer a flexible, scalable, and sustainable approach to biomass separation, maintaining high-quality biomass integrity, and significantly contributing to the economic feasibility and environmental sustainability of biomass valorization strategies.

Precipitation Techniques:

Precipitation techniques are instrumental in the selective extraction and recovery of nutrients and bioactive compounds from biomass suspensions. By adjusting parameters such as pH or introducing specific salts or organic solvents, compounds like polyphosphates, pigments, and vitamins can be effectively isolated for subsequent valorization. For instance, chemical precipitation methods, such as the formation of struvite (magnesium ammonium phosphate), are employed to recover phosphorus from wastewater streams, facilitating its reuse as a fertilizer. Similarly, pigments from microalgae, including phycobiliproteins, can be extracted using techniques like ultrasound-assisted extraction, enhancing yield and purity. These precipitation strategies not only enable the efficient recovery of valuable compounds but also contribute to sustainable biomass processing by minimizing waste and promoting resource recycling[32,33].

Drying Methods:

Biomass drying is a critical step for ensuring long-term preservation and stability, particularly for applications in bioenergy, pharmaceuticals, and food industries. Effective

drying not only extends shelf life but also stabilizes bioactive compounds, reduces microbial activity, and lowers biomass weight, which improves handling and reduces transportation costs. Among the most effective methods for biomass drying are spray drying and freeze drying, each of which offers distinct benefits depending on the nature of the biomass and its intended application.

Spray drying is widely recognized for its rapid moisture removal capabilities and scalability, making it an attractive option for handling large volumes of biomass. In this method, the liquid biomass feed is atomized into fine droplets, which are exposed to a controlled stream of heated air. As the droplets travel through the drying chamber, moisture evaporates rapidly, and dry biomass particles are collected downstream. The rapid drying process minimizes exposure time to high temperatures, thereby reducing the risk of thermal degradation. This is particularly advantageous for biomass with moderate heat sensitivity. Studies have shown that spray drying conditions optimized at airflow temperatures between 110–150°C can effectively reduce nutrient loss while achieving moisture content below 5%, a level that is ideal for biomass stability during long-term storage. Additionally, the incorporation of stabilizing agents such as maltodextrin or gum arabic can improve the structural integrity of bioactive compounds, reducing their susceptibility to oxidation and degradation during drying[34].

Despite its advantages, spray drying has certain limitations, particularly when handling highly heat-sensitive materials such as probiotics or antioxidant-rich extracts. In such cases, freeze drying is often the preferred method due to its superior preservation capabilities. Freeze drying, also known as lyophilization, operates by freezing the biomass and then subjecting it to a vacuum environment where frozen water sublimates directly from solid to vapor, bypassing the liquid phase entirely. This sublimation process occurs at low temperatures, effectively minimizing thermal degradation and preserving delicate cellular structures and volatile compounds. As a result, freeze-dried products typically retain their original texture, color, and bioactive profile better than those produced by spray drying. However, freeze drying is generally slower and more energy-intensive, making it less practical for large-scale biomass processing without careful optimization.

Comparing the two methods highlights important differences in moisture content control, nutrient retention, and energy efficiency. While spray drying is effective for achieving low moisture content in bulk biomass, freeze drying excels in preserving bioactive molecules and volatile compounds. The choice between the two methods often depends on the specific requirements of the biomass and its intended application.

To overcome the limitations of both methods, innovative hybrid approaches have emerged as effective alternatives. For example, microwave-assisted freeze drying accelerates ice sublimation, significantly reducing drying times while preserving thermally sensitive compounds. Similarly, infrared-assisted spray drying enhances heat transfer efficiency, improving drying performance while minimizing heat damage. Furthermore, combining spray drying as a pre-drying step followed by freeze drying has

shown promising results for improving energy efficiency while maintaining superior product quality in high-value biomass materials. These hybrid approaches are gaining increasing attention as the demand for improved biomass drying techniques continues to grow.

3.2.2. Separation Techniques Specific to MF3 Biomass

MF3 biomass, enriched with polyphosphates, pigments, vitamins, and sulfur, requires specialized separation methods to preserve nutrient integrity. Effective separation processes are critical to ensure minimal degradation of these bioactive components, which are particularly susceptible to oxidation or environmental instability.

3.2.2.1. Microbial Pellet Separation

Centrifugation is the preferred technique for microbial pellet separation due to its ability to rapidly concentrate microbial biomass without compromising cellular structure. Microbial pellets enriched in polyphosphates and vitamins are particularly sensitive to prolonged exposure to environmental conditions, requiring prompt and efficient separation to maintain their nutritional value. Centrifugation offers a high recovery rate by applying centrifugal forces that promote rapid sedimentation, effectively concentrating biomass even in dilute suspensions. Studies have demonstrated that centrifugation speeds between 5,000 and 10,000 rpm efficiently separate microbial biomass while minimizing shear stress and preserving cellular integrity. Integrating centrifugation with downstream processes such as filtration or drying can enhance overall biomass stability and streamline production processes.

3.2.2.2. Sulfur Content Preservation

To ensure the preservation of sulfur content in MF3 biomass, anaerobic conditions are highly recommended during separation processes. Sulfur is particularly prone to oxidation when exposed to oxygen, which can reduce its potential as a biofertilizer. To mitigate this, filtration systems combined with nitrogen purging have shown significant success in minimizing sulfur oxidation. By maintaining an oxygen-free environment, sulfur's elemental state is better preserved, enhancing its suitability for agricultural and biotechnological applications. This approach is particularly important for MF3 biomass, where sulfur oxidation can otherwise diminish product efficacy.

3.2.2.3. Bulk Biomass Isolation

For bulk biomass isolation, membrane-based filtration is widely employed due to its ability to achieve high selectivity and efficient recovery. Membrane filtration techniques such as microfiltration (MF) and ultrafiltration (UF) are particularly advantageous for preserving valuable bioactive compounds during biomass recovery. The choice of membrane pore size is crucial in ensuring optimal performance, as pore sizes must match the biomass particle size to maximize separation efficiency. Studies have demonstrated that MF and UF processes provide superior recovery rates with minimal

bioactive compound loss, making them ideal for preserving MF3 biomass properties during separation[35].

In conclusion, by adopting a combination of centrifugation, anaerobic filtration strategies, and membrane-based filtration techniques, MF3 biomass can be effectively recovered while ensuring the preservation of its rich nutrient profile. These tailored separation approaches address the unique challenges posed by MF3 biomass and ensure its potential for application in biofertilizer production, pharmaceuticals, and environmental remediation.

3.2.3. Sustainability and Eco-Friendly Methods

The adoption of sustainable and eco-friendly methods in biomass processing is crucial for minimizing environmental impacts while ensuring economic feasibility. Innovative strategies such as bio-based flocculants, energy-efficient drying, and solvent recovery systems have emerged as effective solutions in this regard.

Bio-based flocculants derived from natural sources such as plant polysaccharides, microbial biopolymers, and starch-based compounds have gained increasing attention for promoting environmentally sustainable separation processes. These flocculants offer several advantages, including reduced chemical input, lower toxicity, and improved biodegradability. By facilitating the aggregation of suspended biomass particles, bio-based flocculants enhance sedimentation and improve separation efficiency while minimizing the presence of residual contaminants. Studies have demonstrated their effectiveness in biomass recovery processes, particularly in wastewater treatment systems and fermentation biomass separation[36].

In drying processes, the adoption of energy-efficient techniques such as low-temperature vacuum drying has proven effective in minimizing energy consumption while preserving the nutritional and bioactive properties of biomass. Low-temperature vacuum drying reduces the thermal impact on biomass by operating under reduced pressure, which lowers the boiling point of water and enables moisture removal at lower temperatures. This method has shown particular promise for preserving temperature-sensitive compounds in food and pharmaceutical biomass products[34].

In addition, environmentally friendly solvent recovery systems are increasingly being implemented to reduce solvent waste and improve process sustainability. Closed-loop evaporative processes allow efficient solvent recovery by continuously recycling solvents during biomass extraction and purification stages. This approach not only minimizes environmental emissions but also improves cost-efficiency by reducing solvent consumption and disposal costs. Studies have shown that solvent recovery systems based on closed-loop designs can effectively recover up to 90% of volatile solvents in biomass processing industries, supporting both environmental and economic sustainability.

3.2.4. Analytical Techniques for Composition Verification

Accurate analysis of biomass composition is essential for characterizing its nutritional profile, bioactive content, and application potential. Various advanced analytical techniques are employed to verify the composition of MF3 biomass, with particular focus on sulfur content, vitamins, pigments, and thermal stability. The integration of these techniques ensures precise characterization, guiding both biomass processing strategies and product quality control.

3.2.4.1. ICP-OES for Elemental Sulfur

Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) is a highly reliable and sensitive method for quantifying elemental sulfur content in biomass samples. This technique utilizes an inductively coupled plasma to excite atoms, which then emit characteristic wavelengths of light that are measured to determine sulfur concentration. ICP-OES is particularly suitable for biomass characterization due to its ability to simultaneously quantify multiple elements with high precision and accuracy. Studies have demonstrated that ICP-OES efficiently quantifies sulfur content even in complex matrices, making it a valuable tool for assessing MF3 biomass enriched with sulfur-rich phototrophic bacteria. This analysis is critical for biomass designated for biofertilizer applications, where accurate sulfur content measurement is essential to ensure soil nutrient balance and optimal agricultural performance[37].

3.2.4.2. LC-MS/UPLC-MS for Vitamins and Pigments

Liquid Chromatography-Mass Spectrometry (LC-MS) and Ultra-Performance Liquid Chromatography-MS (UPLC-MS) provide highly sensitive and specific methods for analyzing bioactive compounds such as vitamins and pigments. These techniques enable the precise quantification of both primary and secondary metabolites, which are crucial for determining biomass quality and stability. The advanced separation capabilities of LC-MS and UPLC-MS allow for the identification of complex pigment profiles, including carotenoids, bacteriochlorophylls, and bioactive polyphenols. In the case of purple and green sulfur bacteria biomass, LC-MS is particularly effective in detecting vitamins like cobalamin, as well as key pigments such as bacteriochlorophyll a and okenone. These bioactive compounds are critical for determining the potential of biomass in biofertilizer applications or as antioxidant-rich food additives. Due to their high sensitivity, LC-MS and UPLC-MS techniques are ideal for detecting trace-level compounds, ensuring comprehensive profiling of bioactive constituents[38].

3.2.4.3. Thermogravimetric Analysis (TGA)

Thermogravimetric Analysis (TGA) is a powerful technique employed to assess biomass stability, moisture content, and thermal degradation behavior. TGA measures the weight loss of a biomass sample as a function of temperature under controlled heating conditions, providing insight into the composition and thermal resilience of biomass materials. For MF3 biomass applications, TGA is particularly useful in determining moisture content, which directly influences shelf life and storage stability. Moreover, TGA helps evaluate the thermal stability of sulfur-rich biomass, ensuring that critical bioactive compounds remain intact during processing or drying steps. This technique is essential

in designing optimized biomass storage strategies that preserve bioactive integrity while minimizing degradation risks. The information gained from TGA allows for improved process control, ensuring product consistency in biofertilizer formulations and other biomass-based applications[39].

3.2.4.4. Integrative Analytical Strategies

In practice, combining these analytical techniques often provides the most comprehensive insight into biomass composition. For instance, ICP-OES can verify sulfur enrichment in biomass, while LC-MS/UPLC-MS allows detailed profiling of vitamins and pigments. Meanwhile, TGA offers thermal stability assessment, which is particularly important when scaling biomass processing to industrial levels. By integrating these analytical methods, biomass producers can ensure product quality, maximize bioactive content retention, and support compliance with regulatory standards for biofertilizer or food-grade biomass applications.

3.2.5. Scaling-Up Considerations

Scaling up biomass separation processes from laboratory to industrial levels requires careful consideration of efficiency, cost-effectiveness, and compliance with safety and environmental standards. The development of scalable methodologies ensures that biomass processing remains viable for commercial applications such as biofertilizer production, bioenergy, and pharmaceutical materials. Successful scale-up strategies integrate robust separation technologies, minimize operational costs, and adhere to industry regulations to guarantee product quality and consistency.

3.2.5.1. Process Efficiency

Efficient biomass separation at an industrial scale depends on the selection of robust and rapid methods capable of processing large volumes while maintaining product integrity. Techniques such as centrifugation and membrane filtration are particularly suitable for large-scale applications due to their adaptability and ability to deliver high throughput. Centrifugation, in particular, enables the rapid concentration of microbial biomass while minimizing exposure to environmental stressors that could compromise bioactive compounds. Industrial centrifuges equipped with automated control systems can efficiently manage continuous feed streams, optimizing both throughput and recovery rates.

Membrane filtration, including microfiltration (MF) and ultrafiltration (UF), has also emerged as a scalable solution for separating biomass from process liquids. These techniques offer selectivity tailored to biomass particle size, ensuring minimal loss of bioactive compounds. In large-scale operations, integrating cross-flow membrane systems improves efficiency by enhancing membrane longevity and reducing fouling, both of which are critical in maintaining consistent productivity across extended production cycles[40].

3.2.5.2. Cost-Effectiveness

To maintain economic feasibility, industrial-scale biomass processing requires the adoption of cost-effective methodologies that minimize energy consumption, chemical usage, and equipment maintenance. The use of renewable flocculants, derived from plant or microbial sources, represents a promising solution for reducing costs while maintaining environmental sustainability. Bio-based flocculants enhance biomass separation by promoting particle aggregation without the need for synthetic chemicals, thereby lowering both material costs and environmental impact. Additionally, energy-efficient drying technologies such as low-temperature vacuum drying can reduce energy consumption during biomass stabilization, further improving cost-efficiency[34].

Process automation is another critical aspect of improving cost-effectiveness. Automated centrifuges, filtration systems, and solvent recovery units allow real-time monitoring of separation efficiency, improving precision while reducing labor costs. By minimizing human intervention, automation ensures consistent performance, optimizing biomass recovery yields.

3.2.5.3. Compliance Standards

Ensuring compliance with agricultural, environmental, and industrial regulations is vital during the scale-up of biomass separation processes. For biomass intended for use in biofertilizer production or greenhouse applications, separation processes must meet stringent requirements regarding purity, nutrient retention, and the absence of harmful residues. This requires the integration of quality control steps at key stages of the separation process.

For example, ICP-OES analysis can verify sulfur content in biomass, ensuring sulfur-enriched biofertilizers meet agricultural standards. Similarly, LC-MS or UPLC-MS techniques are employed to ensure that pigment and vitamin concentrations meet biofertilizer quality requirements. Thermogravimetric analysis (TGA) is frequently employed to assess biomass stability and thermal properties, ensuring that products remain consistent during extended storage and distribution periods[39].

In addition to product purity, environmental compliance is a growing concern for biomass processing industries. Closed-loop solvent recovery systems, emission control technologies, and water recycling mechanisms are increasingly integrated into large-scale processes to ensure environmental sustainability while maintaining operational efficiency.

By adopting scalable separation strategies that combine efficient technologies, cost-effective solutions, and robust quality control frameworks, biomass producers can successfully meet the demands of industrial production while maintaining product quality, safety, and environmental responsibility.

3.2.6. Critical Evaluation and Recommendations

A comprehensive assessment of biomass separation and drying methods reveals distinct advantages and limitations across various techniques. While centrifugation,

membrane filtration, and drying strategies each offer unique benefits, their effectiveness varies depending on biomass composition, processing conditions, and desired end-product characteristics.

3.2.6.1. Centrifugation

Centrifugation has demonstrated remarkable efficiency in rapidly recovering microbial pellets and biomass concentrates. Its ability to effectively sediment microbial cells, polyphosphate-enriched biomass, and nutrient-dense materials makes it a preferred choice for MF3 biomass processing. However, centrifugation is inherently energy-intensive, particularly at industrial scales where continuous operation demands significant power input. While this technique provides unmatched speed and separation precision, its environmental and economic impacts must be considered when scaling up operations. Integrating automated control systems and optimizing rotor speeds can help mitigate energy demands while maximizing recovery rates.

3.2.6.2. Membrane Filtration

Membrane filtration offers an effective compromise between quality preservation and scalability. Techniques such as microfiltration (MF) and ultrafiltration (UF) are particularly advantageous for isolating bulk biomass while preserving delicate bioactive compounds. Membrane filtration minimizes shear stress, ensuring improved retention of vitamins, pigments, and polyphosphate structures in MF3 biomass. However, achieving optimal performance requires precise pore size selection to ensure selective passage of water and low-molecular-weight compounds while retaining valuable biomass components. Additionally, membrane fouling remains a potential challenge, requiring regular cleaning cycles or anti-fouling strategies to maintain efficiency. Despite these limitations, membrane filtration stands out as a sustainable and cost-effective method for large-scale biomass separation when properly managed.

3.2.6.3. Drying Techniques

Low-temperature drying methods provide superior preservation of bioactive compounds, ensuring minimal degradation of nutrients and pigments. Techniques such as vacuum drying and freeze drying reduce thermal exposure, maintaining the functional and nutritional properties of MF3 biomass. Although slower than traditional high-temperature drying methods, these energy-efficient techniques are particularly suitable for preserving antioxidant-rich compounds, sulfur-enriched components, and thermally sensitive vitamins. Careful control of drying parameters, such as pressure reduction and drying cycle duration, can improve both drying efficiency and product stability.

3.2.7. Recommendations for MF3 Biomass Processing

To ensure the optimal processing of MF3 biomass for agricultural and biofertilizer applications, a combined strategy is recommended:

- Centrifugation should be employed for the rapid recovery of microbial pellets enriched with polyphosphates and vitamins. This ensures efficient separation while minimizing the risk of nutrient degradation.
- Membrane Filtration is recommended for isolating bulk biomass while preserving bioactive compounds and nutrients critical for agricultural value. Selecting appropriate pore sizes and integrating anti-fouling measures will maximize both yield and purity.
- Low-Temperature Drying Techniques should be prioritized to stabilize the biomass while preserving its nutritional integrity. Freeze drying or low-temperature vacuum drying techniques are particularly beneficial for preserving sulfur content and antioxidant compounds.

By implementing this integrated approach, biomass producers can effectively balance efficiency, sustainability, and product quality, ensuring MF3 biomass achieves its full potential in agricultural applications while maintaining cost-effectiveness and environmental responsibility.

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