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REVIEW

Use of in situ solid-phase adsorption in microbial natural product fermentation development

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Abstract It has been half a century since investigators first began experimenting with adding ion exchange resins during the fermentation of microbial natural products. With the development of nonionic polymeric adsorbents in the 1970s, the application of in situ product adsorption in bioprocessing has grown slowly, but steadily. To date, in situ product adsorption strategies have been used in biotransformations, plant cell culture, the production of biofuels, and selected bulk chemicals, such as butanol and lactic acid, as well as in more traditional natural product fermentation within the pharmaceutical industry. Apart from the operational gains in efficiency from the integration of fermentation and primary recovery, the addition of adsorbents during fermentation has repeatedly demonstrated the capacity to significantly increase titers by sequestering the product and preventing or mitigating degradation, feedback inhibition and/or cytotoxic effects. Adoption of in situ product adsorption has been particularly valuable in the early stages of natural product-based drug discovery programs, where quickly and cost-effectively generating multigram quantities of a lead compound can be challenging when using a wild-type strain and fermentation conditions that have not been optimized. While much of the literature involving in situ adsorption describes its application early in the drug development process, this does not imply that the potential for scale-up is limited. To date, commercial-scale processes utilizing in situ product adsorption have reached batch sizes of at least 30,000 l. Here we present examples where in situ product adsorption has been used to improve product titers or alter the ratios among biosynthetically related natural products, examine some of the relevant variables to consider, and discuss the mechanisms by which in situ adsorption may impact the biosynthesis of microbial natural products.

Keywords In situ adsorption · Natural product fermentation · Solid-phase adsorption · Adsorbent resin

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Introduction

Natural products have historically provided a rich source of lead compounds for drug discovery. Fully 27 % of all new chemical entities approved by the United States Food and Drug Administration from 1981 to 2006 were either natural products or semisynthetic derivatives of natural products. This includes 68 % of new antibacterial and 34 % of new anticancer agents [53]. Other clinical indications where natural products have yielded prominent therapeutics include lipid disorders (e.g., statins) and immunosuppression (e.g., cyclosporine-class, rapamycin-class). From a business perspective, natural product drug discovery has declined as many pharmaceutical companies have shifted their focus from acute diseases, such as infection, to chronic diseases that have a greater potential to yield "blockbuster" drugs. Perhaps more importantly, however, are some of the real and perceived technical challenges often cited as precipitating the shift from natural product-based drug discovery and development to synthetic alternatives [11, 24, 53]. These include difficulty adapting

natural product-based libraries to high-throughput screening technologies, diminishing returns from existing natural product libraries in traditional therapeutic areas, complications arising from dereplication and challenges in supplying sufficient quantities of natural product to support early stage development. Additionally, the high expectations that combinatorial chemistry and fragment-based drug discovery strategies will provide alternative avenues to chemical diversity have doubtlessly drawn resources away from natural product research.

There is ample reason however, to believe that natural products still have a role to play in drug discovery. The increased incidence of antibiotic resistance in recent years underscores the continuing need to develop new anti-infectives, a therapeutic area where natural products have long dominated. To date, only a single class of totally synthetic antibiotics, fluoroquinolones, has reached clinical use. In addition, the bounty of new therapeutic targets arising from genomics to bioinformatics creates new opportunities for natural products outside their traditional clinical indications. While focused combinatorial chemistry libraries have proven very useful in lead optimization, their record at lead discovery has not lived up to expectations [53]. By contrast, dramatic advances in the speed and cost of genomic sequencing are revealing untapped chemical diversity in the many unexpressed (cryptic) natural product biosynthetic pathways. Efforts to exploit this diversity through genome mining strategies are still in their early stages, but may provide a new source of natural product-derived chemical diversity for drug discovery in the future. The emergence of synthetic biology strategies holds similar potential. All of this underscores the continued need and the benefits of improving strategies for efficient natural product fermentation development.

As mentioned, one of the primary challenges of working with natural product-derived lead compounds is obtaining sufficient quantities of product early in development. A robust early stage medicinal chemistry effort could easily consume 10's of grams of a natural product starting material per month and requirements will likely increase from there as multiple lead compounds enter into preclinical studies. Natural products frequently have complex structures that are not amenable to multi-gram-scale chemical synthesis and the producing organism is often a wild-type strain which initially may only yield a few milligrams per liter, at best, of the desired product when cultured under conditions that may not have been optimized. Strain improvement and fermentation development can certainly alleviate this bottleneck. Historically, however, these efforts can consume time and resources that project managers, pressed to either fail early and cheaply or get to market quickly, may be reluctant to commit to a single early stage lead molecule.

Significant progress has been made in the last two decades to expedite natural product process development. Examples of general advances include the introduction of optical-based pH and dissolved oxygen sensors, permitting the miniaturization of bioreactor systems, and the broader use of statistical design software to help guide bioprocess development at its early stages. One additional tool that can often yield significant increases in natural product fermentation titers in a relatively short span of time (weeks or months) is in situ product adsorption. This technique is not predicated on having any knowledge of the pathways involved in the biosynthesis of the target natural product. Furthermore, evaluating in situ adsorption as a bioprocess option synergizes well with more traditional avenues of bioprocess development, such as strain improvement and fermentation medium optimization; it is therefore not a matter of choosing one approach in lieu of another. For these reasons in situ adsorption is often well suited for application at the critical and challenging early stages of natural product drug development. Beyond its potential utility in supplying much-needed product early in development, in situ product adsorption is also a feasible option for scale-up and commercialization. Indeed, a commercial-scale process utilizing in situ adsorption in conjunction with strain improvement and traditional medium optimization has already been developed for the production of epothilone B. This process has been demonstrated at working volumes of about 30,000 l and is capable of generating over 5 kg of crude product per batch [4].

Here we present examples of in situ product adsorption using nonionic polymeric resins and discuss some of the important variables that may impact the process. Additionally, we consider the various mechanisms relevant to in situ product adsorption and how considering these mechanisms may help identify bioprocesses where in situ adsorption may be particularly effective.

Overview of in situ product recovery

In general, in situ product recovery (ISPR) or extractive fermentation schemes can take many physical forms. Each form of ISPR has its advantages and limitations, which have been the subject of prior reviews [14, 50, 64]. Relevant variables include the choice of the extractive phase, whether or not the extractive phase comes into direct contact with the cell and whether the extraction is carried out inside the fermentor or as part of a more complex external recirculation loop. Gas stripping, for example, is limited to volatile products. The addition of immiscible solvents or liquid polymers can adversely affect cell growth and viability, impair oxygen transfer and result in troublesome emulsions or otherwise be difficult to separate

from the aqueous phase at the end of fermentation [26, 64]. Systems involving external recirculation loops usually entail increased cost and complexity, additional challenges in maintaining sterility and heterogeneous mixing/aeration within the system [50].

By comparison, the addition of a solid adsorbent directly into the fermentor is probably the least restrictive form of ISPR for most applications. However, while this approach may alleviate some of the issues cited above, it is not entirely free of drawbacks and constraints. As with liquid extractive phases, solid adsorbents may sequester critical nutrients and may adversely impact cell growth and primary metabolism. Depending on growth morphology, difficulties may also arise in separating solid-phase adsorbents at the end of fermentation. Concerns have also been raised about their potential to act as abrasives on tank surfaces and mechanical agitator seals with prolonged use [64]. Due to its simplicity and ease of implementation, however, solid-phase in situ adsorption appears to be the most commonly employed form of ISPR.

Aside from its utilization in natural product fermentation within the pharmaceutical industry, solid-phase in situ product adsorption has been investigated in a variety of other bioprocesses. These include the production of natural products from plant cell culture [58, 62, 71], precursor directed biosynthesis schemes [40], as a means for dosing poorly soluble substrates and minimizing product inhibition in biotransformations [3], the production of certain commodity chemicals such as lactic acid [51] and phenylalanine [35, 36], the production of second generation biofuels such as butanol [57] and in the enrichment of natural product libraries at the discovery stage [20, 34, 44]. While these examples lie beyond the principle scope of this review, they do provide relevant lessons and further validate the utility of using solid-phase adsorption to sequester natural products during fermentation.

Evolution of adsorbent resin technology

Solid-phase adsorption has been used for many years as a primary recovery method at the end of fermentation as well as in subsequent purification steps. The first adsorbents were synthetic ion exchange resins and natural adsorbents, such as activated charcoal. The practice of adding adsorbents during fermentation dates back to at least 1959, when ion exchange resins where added to fermentations of neomycin and novobiocin [10]. While these early examples of in situ adsorption succeeded in binding the product, they did not actually result in improved titers. Other practical issues hindered the application of in situ adsorption in fermentation. Chief among these was the limited spectrum of commercially available adsorbents. Activated charcoals have found few in situ applications because particles lack the mechanical strength to withstand agitation and products are often difficult to elute [13, 42]. Ion exchange resins tend to bind polar nutrients along with polar natural products which may both alter the fermentation medium and result in lower binding capacities. Anion exchangers may additionally bind to cells which present negative charges [67]. While ion exchange resins are still an option for in situ applications, their use in natural product fermentation has been limited to removing charged inhibitory compounds other than the product of interest, such as lactic acid and ammonia [54, 75].

The 1970s saw the introduction of macroporous nonionic polymeric adsorbent resins that are now used in the majority of applications of in situ adsorption in fermentation [1]. A partial list of adsorbents suitable for in situ application is included here, along with a summary of their physical properties (Table 1). Most of the commonly used nonionic adsorbent resins are composed of a polystyrenic backbone crosslinked with divinylbenzene, giving them a hydrophobic character. In general, they have a high affinity for hydrophobic and aromatic compounds, binding these metabolites by hydrophobic interaction or π–π bonding [13]. Many functionalities, such as, hydroxyl, amino and cyano groups have since been bound to this polystyrenic scaffold, resulting in altered binding selectivity. However, most of these functionalized polystyrenic adsorbents are geared toward chromatographic applications and are too expensive and have particle sizes too small to be readily recovered from fermentation broth. For binding less hydrophobic metabolites the principle alternatives are the non-aromatic acrylic ester-based polymeric adsorbents such as XAD-7 (Rohm and Hass—Dow Chemical) and HP2MG (Mitsubishi). Aside from distinctions in polymer chemistry, adsorbent resins are typically differentiated by bead size, pore size, surface area and density. Nonionic polymeric adsorbents have an additional advantage in that elution is typically done in organic solvents that can be readily evaporated to reduce processing volumes and are often more easily integrated with subsequent isolation steps than the aqueous elution buffers used with ion exchange.

Application of in situ adsorption in fermentation processes

Within the context of early stage natural product drug development in the pharmaceutical industry, there are many examples of in situ product adsorption being successfully used to increase product titers. This list includes a variety of clinically relevant natural products derived from bacterial and fungal fermentation (Table 2). In addition, in situ adsorption has been cited in the discovery of a

Table 1 Summary of properties of selected (currently marketed) nonionic polymeric adsorbents suitable for in situ applications

Name	Manufacturer	Polymer chemistry	Particle size (um)	Sieve size[b]	Surface area (m^2/g)	Pore size (Å)
HP20	Diaion (Mitsubishi)	Styrenic-DVB	300–1,180	60	590	290
HP2MG	Diaion (Mitsubishi)	Methacrylate	300–1,180	50	570	240
SP207	Diaion (Mitsubishi)	Styrenic-DVB-Br[a]	300–1,180	50	600	110
SP850	Diaion (Mitsubishi)	Styrenic-DVB	>250	60	930	45
SP825L	Diaion (Mitsubishi)	Styrenic-DVB	>250	60	930	70
SP700	Diaion (Mitsubishi)	Styrenic-DVB	>450	40	1,200	90
Lewatit VP OC 1064	Lanxess	Styrenic-DVB	440–540	40	800	50–100
Lewatit VP OC 1062	Lanxess	Styrenic-DVB	450–550	40	600	500–1,000
Lewatit OC 1600	Lanxess	Styrenic-DVB	315–1,000	50	130	150
Optipore L493	Dow Chemical	Styrenic-DVB	300–840	50	≥1,100	46
FPX66	Dow Chemical	Aromatic Polymer	600–750	30	≥700	nd
XAD7 HP	Rohm Haas (Dow)	Aliphatic Acrylic	560–710	35	≥500	450
XAD1600 N	Rohm Haas (Dow)	Styrenic-DVB	350–450	45	≥800	150
XAD4	Rohm Haas (Dow)	Styrenic-DVB	490–690	35	≥750	100
XAD18	Rohm Haas (Dow)	Styrenic-DVB	375–475	45	≥800	150
XAD761	Rohm Haas (Dow)	Formophenolic	560–760	35	≥200	600
XAD16 N	Rohm Haas (Dow)	Styrenic-DVB	560–710	35	≥800	150
XAD1180 N	Rohm Haas (Dow)	Styrenic-DVB	350–600	45	≥500	400
X-5	Anhui sanxing resin tech	Polystyrene	300–1,250	50	500–600	290–300

[a] Brominated

[b] U.S. standard sieve size to retain beads at the lower end of the cited particle size range

number of novel natural products, indicating its application at the earliest stages of drug discovery (Table 3). As is evident, these lists comprise a range of natural product classes and producing organisms. Also highlighted (Table 2) are the principle mechanisms responsible for titer enhancement and three variables to consider when employing solid-phase adsorption during fermentation: (1) what resin to use, (2) how much resin to add, and (3) when to add it.

The principle process variables and the fundamental mechanisms of action of in situ adsorption will be further discussed in the following sections, with examples which hopefully the reader will find helpful and relevant. Additional issues, such as the physical and functional integrity of the adsorbent will also be discussed. First and foremost among these is the impact in situ adsorption may have on making accurate measurements of product titers. Sample preparation methods developed for extracting a natural product from a fermentation broth in the absence of an adsorbent may be inadequate when it comes to extracting the same product when bound to a resin. Reassessing sample preparation methods at the earliest stage of evaluating in situ product adsorption is a critical step. Complications may also arise if the solid resin particles settle rapidly in the fermentation broth, resulting in a heterogeneous sample. Conversely, it may be difficult to separate the resin from the mycelium. In this case it may be easier to recover the resin, cell-solids and insoluble medium components by centrifugation and extract them together as a single solid phase. Assessing complete extraction of the product from a solid adsorbent can be easily accomplished by assaying product titers from successive extractions of the same solid sample. One also cannot assume that all of the product will be bound to the resin. Failure to ensure robust extraction methods could result in an underestimation of product titers and missed opportunities for process improvements.

Impact of screening different adsorbent resins

Adsorbents considered for in situ adsorption in fermentation may vary in chemical and physical properties such as, polymer chemistry, surface area, particle size and pore size (Table 1). Different combinations of properties can significantly alter their performance. Even resins with some similar characteristics may give differing results. Instances where multiple resins were individually evaluated illustrates that it is often worth the effort to screen multiple adsorbents. For example, when four adsorbents (XAD-2, XAD-7, XAD-8 and HP20) were evaluated for their impact on the production of dynemicin A, titer increases ranged from 2 to 5-fold [38]. In the case of trichodimerol (BMS-182123) production from *Penicillium chrysogenum,* four out of five nonionic polymeric adsorbents (XAD-7, XAD-8, XAD-16 and HP20)

Table 2 In situ solid-phase adsorption of natural products in bacterial and fungal fermentation

Natural product	Producing organism	Adsorbent	Time of addition	Titer (mg/l) without → with in situ adsorpt.[a]	Cited/proposed mechanism of action	Vol. (l) [b]	Ref.
Streptovaricin C	*Streptomyces spectabilis*	10 % HP20	Inoculation	8 → 99 (2,000)	Degradation	18	[27, 74]
Trichodimerol	*Penicillium chrysogenum*	1 % XAD-8	24 h	15.3 → 86.3	Degradation	Flasks	[70]
Epothilone D	*Myxococcus xanthus* (heterologous expression)	2 % XAD-16	Inoculation	0.16 → 0.45 (20–30)	Degradation	1,000	[2, 16, 39]
Salinosporamide A	*Salinispora tropica*	2 % XAD-7	24 h	5.7 → 278	Degradation	Flasks	[66]
Leinamycin	*Streptomyces* sp.	5 % HP20	18 h	3.0 → 33	Degradation	1,000	[22]
Dynemicin A	*Micromonospora chersina* (ATCC 53710)	1 % XAD-8	72 h	3.6 → 24.7	Degradation autotoxicity	Flasks	[38]
Paulomycin A, B	*Streptomyces paulus*	0.9 % XAD-2	Inoculation	40 → 380	Degradation autotoxicity	Flasks	[46]
Esperamicin A1	*Actinomadura verrucosopora* (ATCC 3933)	1 % HP20	Inoculation	5.3 → 8.1	Degradation autotoxicity	Flasks	[37]
Pristinamycin	*Streptomyces pristinaespiralis* XC505	12 % JD-1	20 h	360 → 800	Degradation autotoxicity	1.5	[5, 68]
Teicoplanin	*Actinoplanes teicomyceticus* (mutant)	5 % HP20	Inoculation	32 → 134 (1,500)	Degradation autotoxicity	300	[41, 42]
Cyclohexamide	*Streptomyces griseus*	6 % XAD-7	48 h	700 → 1,300	Degradation feedback inhibit.	10	[69]
Nargenicin A1 Nodusmicin	*Saccharopolyspora* sp.	3 % HP20	Inoculation	27 → 202 157 → 309	Degradation feedback inhibit.	500	c
Soraphens A1α	*Sorangium cellulosum* Soce 539	2 % XAD-1180	Inoculation	50 → 100 (230)	Degradation feedback inhibit.	4,700	[19, 20]
FK506 FK520	*Streptomyces* sp. GT1005	5 % HP20	Inoculation 1 day	100 → 356 2.3 → 8.7	Feedback inhibit.	Flasks	[33]
Beauvericin	*Fusarium redolens* Dzf2 (endophytic fungi)	5 % X-5	4-5 days	179 → 264	Feedback inhibit.	Flasks	[73]
Cercosporamide	unidentified fungal strain (LV-2841)	3 % HP20 1 % XAD7	Inoculation	≈10 → 300–500	Autotoxicity	10	[63]
Rubradirin	*Streptomyces achromogenes v. rubradiris*	6 % HP21	24 h	5–10 → ≈50 (250)	Autotoxicity	250	[47]
Kirromycin	*Actinoplanes* sp. A8924	3 % S-112	Inoculation	300–350 → 1,200	Autotoxicity	3	[18]
Epothilone B	*Sorangium cellulosum* So ce90	0.8 % FPC22	120 h	5.2 → 14.3	Binding $NH4^+$	Flasks	[54]
Epothilone B	*Sorangium cellulosum* So ce90B2 (mutant ATCC PTA-3880)	≈2 % XAD-16	Inoculation	(≈170 mg/l)	None Cited	30,000	[4]

Table 2 continued

Natural product	Producing organism	Adsorbent	Time of addition	Titer (mg/l) without → with in situ adsorpt.[a]	Cited/proposed mechanism of action	Vol. (l)[b]	Ref.
Nisin	*Lactococcus lactis* (Tn*Nip*)	IRA-67	Added as pH control	2-fold increase	Lactate Removal	1	[75]
Prodigiosin-like pigment	*Serratia* sp. KH-95	10 % HP20	12 h	5.3 → 6.9	None Cited	Flasks	[32]
Verrucarin A Verrucarin J	*Mycothecium verrucaria* CL-72	8.3 % XAD-7	Inoculation	700 → 1,477 357 → 536	None Cited	Flasks	[28]
Thailandepsin A	*Burkhoderia thailandensis*	4 % HP20	12 h	83 → 158	None Cited	Flasks	[43]

[a] Reflect product titers without and "→" with in situ adsorption under identical fermentation conditions. Titers given in () reflect in situ adsorption in combination with additional fermentation development and/or mutagenesis

[b] Volumes are working volumes. Volumes represent the largest volume where in situ adsorption was employed, but this volume does not necessarily correlate to the titers that are presented, which may have been achieved in flasks or smaller fermentors

[c] T. Phillips et al. manuscript in preparation

increased titers from 2 to 4-fold while one of the resins tested (XAD-2) decreased titers 5-fold [70]. At a minimum, evaluating resins of differing polymer chemistries and polarities (e.g., Table 1, methacrylate vs. polystyrenic) may be a good starting point. In addition to criteria such as product titer and metabolite profiles, the ease and selectivity of elution of the product from the adsorbent may also influence ones choice.

The screening of adsorbents can typically be performed in shaking flasks or small parallel fermentor systems, with only the best performing adsorbent(s) being subsequently scaled up to lab-scale fermentors. If time and circumstances permit, evaluating multiple adsorbents in several different fermentation media may provide more complete information for subsequent development. As with fermentation medium development, results from shake flasks do not always translate to the fermentor. Consider the production of kirromycin by *Actinoplanes* sp. A8924, where the addition of 5 % SP-205 polymeric adsorbent (Mitsubishi) to shake flask cultures increased titers from 360 to 1,250 mg/l, while the addition of 5 % S-112 (Dow Chemical Co.) only increased titers to 960 mg/l [18]. When the same comparison was made under optimized conditions in fermentors, the order of the adsorbents was reversed, with the S-112 adsorbent increasing titers from 400 to 1,500 mg/l, while the SP-205 adsorbent achieved a maximum titer of only 700 mg/l. The reason for this reversal was that under shake flask conditions both adsorbents completely bound the product, but in a fermentor, the SP-205 adsorbent only bound about 57 % of the product. Exactly why the SP-205 adsorbent did not completely bind the product in the fermentor was not determined.

The preceding examples demonstrate the advantages of screening different resins in the context of increasing titers of a target natural product from a particular strain. To the best of our knowledge the ability of different resins to elicit qualitatively different natural product profiles across many strains has not been rigorously investigated or at least reported in the literature. The value of including different resins as variables in the creation of natural product libraries would have to be weighed against the value of incorporating other variables such a temperature and fermentation medium. One possible means of incorporating multiple resins into a natural product discovery protocol without increasing the total number of variables would be add two or more resins in combination.

Impact of resin concentration

The concentration of the adsorbent, usually measured on a weight per volume basis, must also be experimentally optimized. Resin concentrations cited in the literature range from 0.8 % to as high as 20 %. Adding too little adsorbent,

Table 3 Selected sample of *In-situ* adsorption in natural product discovery

Natural product	Producing organism	Adsorbent	Time of addition	Volume (l)	Ref.
Tiacumicin B	*Dactylosporangium aurantiacum hamdenensis* (NRRL 18085)	XAD-16 (conc. unknown)	No data	5	[61]
Tirandamycin C, D	*Streptomyces* sp. 307-9	XAD-16 (conc. unknown)	48 h	Flasks	[7]
Sapurimycin	*Streptomyces* sp. DO-116	10 % HP-20	24 h	15	[23]
Clecarmycin C	*Streptomyces* sp. DO-114	5 % HP20	18 h	1,100	[17]
Lomaiviticin A, B	*Micromonospora lomaivitiensis*	1 % HP20	Inoculation	70	[25]
Exfoliamycin (and related compounds)	*Streptomyces exfoliates* (Tü-1424)	20 % XAD-1180	36 h	Flasks	[56]
Lymphostin (LK6-A)	*Streptomyces* sp. KY-11783	10 % HP-20	Inoculation	18	[52]
Spiroxins	Fungal Strain LL-37H248	HP20 (conc. unknown)	Inoculation	ND	[49]
Isomigrastatin	*Streptomyces plantensis*	10 % XAD-16	Inoculation	100	[72]
Dorrigocins	*Streptomyces plantensis* Subsp. *rosaceus* AB1981F-75	10 % XAD-16	Inoculation	15	[30]
Jerangolid A	*Sorangium cellulosum* So ce 307	1.7 % XAD-16	Inoculation	60	[21]
Pseurotin A (and related compounds)	*Aspergillus fumigatus*	5 % XAD-16	Inoculation	24	[6]

for example, may inadequately sequester the product of interest, while adding too much may sequester nutrients, negatively impacting cell growth and potentially product titers. In addition, excessive resin concentrations may increase the load of impurities being carried into subsequent isolation steps or increase the volume of solvent required to fully elute a more dispersed resin-bound product.

Increased product titers were found to reach a maximum and then decline with increasing concentrations of adsorbent in the production of a prodigiosin-like red pigment, FK506, dynemicin A, teicoplanin, and cercosporamide [32, 33, 38, 42, 63]. In these examples, product titers peaked at resin concentrations ranging from 1 to 5 %. It is important to note, however, that in all but one of these cases, titers at the highest concentrations of adsorbent were still greater than controls where no resin was added. The notable exception was dynemicin A, where titers actually decreased from 3.6 mg/l in the absence of an adsorbent to zero when the concentration of HP20 was increased to 4 % or higher. By comparison, 1 % HP20 increased dynemicin A titers to 16.2 mg/l [38]. This result highlights the presence of competing mechanisms that can inhibit and enhance natural product titers in an in situ adsorption environment, with the outcome reflecting a balance of these mechanisms. Recognizing the potential for multiple mechanisms to impact product titers can help guide optimization efforts. Not surprisingly, optimum resin concentrations may vary significantly from one resin to another as well. For example, kirromycin titers declined 23 % from their maximum values when the concentration of SP-205 resin (Mitsubishi Chemical Corp.) was increased from 7.5 to 10 %. No decline in titers, however, was observed when concentrations of another resin, S-112 (Dow Chemical Co.), were similarly increased [18]. Titers of paulomycin, rubradirin, and beauvericin all reached a maximum with no decline upon increasing the concentration of adsorbent over the ranges evaluated [46, 47, 73].

Diminishing titers with increasing concentrations of adsorbent is commonly attributed to the sequestering of nutrients in the fermentation medium [32, 36, 38, 54]. Although nonionic hydrophobic adsorbents should not exhibit as strong an affinity for polar medium components as ion exchange resin, it is helpful to recognize their effect on medium composition is not negligible. For example, XAD-16 resin has been shown to bind methyl oleate, the principle carbon source in the production of epothilones by *Myxococcus xanthus* [15]. The addition of HP20 resin resulted in a dose dependent decrease in casein concentrations, as measured using a modified Biuret assay, with 20 % HP20 giving a 55 % decrease in casein concentrations relative to no resin [32]. Aromatic amino acids are particularly prone to binding to polystyrenic adsorbents. In situ adsorption, in fact, has been investigated as a means of enhancing phenylalanine production by *Brevibacterium lactofermentum* [35, 36]. In addition to phenylalanine, XAD-16 was also found to bind tyrosine and "colored components" as measured by optical density (OD_{420}) from the soybean hydrolysate used in the fermentation medium. In one experiment, solutions of soybean hydrolysate were decolorized with various concentrations of XAD-16 adsorbent. When the decolorized soybean hydrolysate was then used in the preparation of the standard fermentation

medium, growth and phenylalanine production by *B. lactofermentum* in the absence of resin were both reduced, with increasing severity correlating to increasing amounts of XAD-16 used in the decolorization [36]. Microorganisms whose growth and natural product titers are very sensitive to medium composition may be particularly sensitive to resin concentration.

Sterilizing fermentation medium components separately from the resin may mitigate the adsorption of nutrients. Consider the production of cercosporamide where the addition of 1 % XAD-7 adsorbent to the medium increased product titers from 10–20 to 500 mg/l. When the resin concentration was increased to 5 % XAD-7, titers dropped 40 % from their maximum values at 1 % XAD-7. This decline in titers, however, was cut in half when the 5 % XAD-7 resin was sterilized apart from the fermentation medium [63]. A similar benefit to sterilizing the adsorbent apart from the fermentation medium was found in the production of rubradirin [47].

While it is helpful to be cognizant of the influence of in situ adsorption on the fermentation medium, no generalizations can be made with regard to the impact of in situ adsorption on cell growth across different natural product producers. Among the references where cell growth was measured, three report no effect on cell growth with the addition of an adsorbent to the medium [16, 19, 22], three present data showing lower cell densities [5, 32, 36] and one cited an increase in cell density with the addition of resin [63]. Lower cell growth may be explained by the sequestering of nutrients, while higher cell growth might be explained by the sequestering of a natural product that is toxic to the producing organism. In many cases the observed impact of in situ adsorption on cell growth may be the net effect of multiple mechanisms, with cell growth in turn influencing product titers. This may be best illustrated by considering the production of pristinamycin, where the addition of 12 % JD-1 adsorbent (Jainyang Pharmaceutical Co.) resulted in a 3-fold increase in titer with a concurrent 22 % decrease in dry cell weight. The decrease in cell density occurred despite the fact that pristinamycin is toxic to the producing strain [5].

As mentioned earlier, the adoption of an in situ adsorption strategy synergizes well with traditional fermentation medium development and the effects of in situ adsorption on the fermentation medium highlights the interaction of the two. The optimum resin concentration may be medium dependent and changing the medium composition to counteract the negative effects of the resin on the medium is as much a viable option as limiting the resin concentration to minimize its effect on the medium. Fed-batch fermentation strategies could also be considered as a means of compensating for nutrients that might bind to the adsorbents.

Impact of timing of resin addition

Another means of minimizing adverse effects of adsorbent resins on the fermentation medium and cell metabolism is to add the adsorbent sometime after the growth phase of the culture. This is standard practice in plant cell culture, where cell growth is more sensitive to the presence of vitamins and growth factors that are readily sequestered by adsorbent resins [58, 62, 71]. The same strategy has also been successfully employed in natural product fermentations involving bacteria and fungi. Returning to the example of pristinamycin, dry cell weights of *Streptomyces pristinaespiralis* decreased from 18 to 12 g/l with the addition of 12 % JD-1 (Jianyang Pharmaceutical Co.) adsorbent when added at the time of inoculation, while product titers increased from 0.4 to 0.6 g/l [5]. When the resin was added 20 h post-inoculation, however, the dry cell weight only decreased to 14 g/l while pristinamycin titers increased to 1.1 g/l. The timing of resin addition in this case corresponds to the onset of pristinamycin biosynthesis. Addition of the adsorbent after 20 h showed a time dependent decrease in both dry cell weight and titers due to the toxicity of pristinamycin towards the producing organism.

With the concentration, timing and type of adsorbent all to consider as independent variables and a myriad of possible two-factor interactions and competing effects as the adsorbent interacts with both products and nutrients, statistically designed experimental methods (DoE) can prove useful in identifying key process parameters. Considering what is known about the structure, stability, bioactivity and biosynthesis of the natural product in question may also provide valuable insight and aid in choosing experimental ranges. For example, a hydrophilic product from a fastidious strain might narrow the types of adsorbents to screen and lead one to opt for lower concentrations and/or later addition times. While one can imagine making a significant time investment to achieve an optimal process, a quick answer as to the potential of in situ adsorption in any particular setting is probably attainable in a single experiment. Four different resins at two concentrations and two different addition times (sixteen shake flasks) would have likely yielded higher product titers for every natural product cited in this review (Tables 2 and 3) More extensive optimization efforts can proceed from there as warranted.

Additional considerations in the application of in situ adsorption

In situ adsorption of cell-associated natural products

One may hypothesize that in situ adsorption will not be effective unless the natural product of interest is exported

from the cell into the fermentation medium. Some natural products may be cell-associated without necessarily being intracellular [8, 60]. Consider the production of FK506 by *Streptomyces* sp., where there is virtually no product detectable in culture supernatants. When 5 % HP-20 was added at the time of inoculation, FK506 titers in the whole broth increased from 100 to 355–365 mg/l, with 95+ % of the product residing on the resin as opposed to the biomass [33]. When this same concentration of resin was enclosed in a miracloth pouch, FK506 titers were the same as control flasks where no resin was added. While this demonstrates that proximity of the cells and resin is important, whether actual cell-resin contact is critical could not be unambiguously determined from these results. It is interesting to consider, however, that in cases where the product is cell-associated, the broth viscosity and growth morphology (pelleted vs. homogeneous) of the producing strain may be important determinants of the effectiveness of in situ product adsorption.

Impact of the medium components on the adsorbent resin

In addition to the impact of the resin on the fermentation medium is the impact of the fermentation medium on the adsorbent. Blinding of adsorbents may occur either by cell debris or insoluble components in the fermentation medium blocking the pores of the resin and thereby reducing the available surface area or by nutrients and other metabolites competing with the product of interest for binding sites on the resin. Either way, the phenomena of decreased binding capacity of the adsorbent occurring over time has been well documented. The binding capacity of XAD-16 resin for epothilone, for example, decreased by 20 % over the course of a 7-day fermentation of *M. xanthus* and 50 % over the course of a 7-day fermentation of *Sorangium cellulosum* [15]. All of the variables (type of resin, concentration of resin and time of addition) discussed to this point can be considered in the context of minimizing any observed blinding of adsorbents.

The propensity for some fermentation medium ingredients to cause foaming in sparged bioreactors can be particularly problematic in processes involving in situ adsorption. In smaller (10–20 l) sparged fermentors a significant fraction of resin particles may be carried into the headspace by foam. This can occur even in fermentors that contain a foam control system as some degree of foaming has to occur before the antifoam system is triggered. Minimizing gas flow rates, altering medium composition to eliminate ingredients that tend to result in foaming, adding antifoam agents proactively to the fermentation medium or adding higher concentrations of adsorbent to compensate for that lost into the headspace are all possible remedies. Addressing foaming issues prior to exploring in situ adsorption may prevent problems during scale-up into sparged bioreactor systems. If antifoam agents are required in sparged fermentors, then it may be prudent to evaluate their impact early in the process of exploring in situ adsorption options (resin screening, etc.). Foaming should also be considered in any subsequent medium development efforts aimed at improving an established in situ adsorption process.

Physical effects of resin

It has been reported that shear effects associated with high concentrations of solid-phase adsorbents (>10 %) are responsible for lower cell growth and streptovaricin titers in cultures of *Streptomyces spectabilis* [74]. While one cannot unequivocally rule out this possibility, as shear effects may be strain dependent, it has not been widely reported or rigorously investigated in a manner that clearly differentiates effects of shear from adsorption of nutrients. The fact that most applications of in situ adsorption are conducted at resin concentrations less than 10 % also limits the availability of corroborating data. The widespread use of other insoluble medium components, such as calcium carbonate, however, would seem to discount significant shear effects of in situ adsorbents for most strains. As discussed below, it has also been estimated that the magnitude of the shear force due to agitation is greater than that associated with the solid resin particles swirling about in the fermentation broth; this would tend to minimize the relative contribution of the latter in creating an environment that is destructive to cells.

Integrity of the resin in an agitated environment

Directly adding solid adsorbent to a well-agitated fermentor also raises some concerns about the effects of agitation on the integrity of resin beads. While the physical integrity of the resin particle may not impact its capacity to sequester the product, it can negatively impact the overall process, as the first step in isolating resin bound products is often to recover the resin on a sieve. The mesh of the sieve must be small enough to retain the resin while large enough to prevent clogging by cell pellets or insoluble medium components. Physically degraded resin particles and the product bound to them may pass through the sieve, constituting a loss to isolation and poor reproducibility.

Addressing the integrity of polystyrenic adsorbent beads in an agitated environment, Frykman and co-workers compared two possible means of resin bead breakage, bead–bead collision and bead-agitator collision [15]. Previously published mathematical models with terms accounting for both the kinetic energy of collision as well as the frequency of collision were used in the analysis. At

impellor speeds of 800 rpm, these models indicate that most of the bead breakage was more likely to be caused by the impellors hitting the beads as opposed to the beads colliding with each other. This relationship between agitation rates and bead breakage was then evaluated experimentally using laser diffraction particle sizing to measure the resin particle size distribution. The evaluation was performed using XAD-16 resin in a water-glycerol solution with an agitation profile designed to mimic the broth viscosity and shear forces of an actual fermentation process. Bead breakage for the first 3 days when the agitation rate was 600 rpm (impellor tip speed = 2.0 m/s) was found to be negligible. After 10 days at 800 rpm (impellor tip speed = 2.7 m/s), however, the particle size was bimodally distributed, with 29 % of particles having a diameter <250 μm and the remaining particles being relatively unchanged (mean diameter of 700 μm).

Larger-scale antibiotic fermentations are typically conducted at impellor tip speeds of 5–7 m/s [29]. As mentioned earlier, the largest fermentation utilizing in situ product adsorption cited here (Table 2) had a working volume of approximately 30,000 l [4]. While no mention was made of impeller tip speeds, bead breakage or the means by which the resin was recovered from the broth, the benefits of adding the adsorbent clearly outweighed the costs in this example. Bead breakage (like cell breakage) is likely to be influenced by other factors such a broth rheology and length of fermentation. The results from Frykman et. al., however, suggest it may prudent to monitor bead breakage upon scale-up and if necessary factor this into the ultimate analysis of any proposed process involving in situ product adsorption.

Mechanisms of in situ product adsorption

It is worth considering how in situ adsorption is impacting titers as it may help identify circumstances where its application may be particularly effective. In situ adsorption may increase natural product titers by a variety of mechanisms including mitigating cytotoxicity of a product towards the producing organism, mitigating cytotoxicity by a metabolite other than the product, preventing product degradation over time and minimizing potential feedback inhibition by the product. Of the twenty examples of in situ product adsorption where a mechanism of action was given (Table 2), preventing product degradation or further metabolism was cited in more than half the cases (65 %). This was followed by mitigating autotoxicity (40 %), minimizing feedback inhibition (25 %) and sequestering an inhibitory metabolite (lactate and ammonia) other than the product of interest (10 %). In some cases multiple modes of action were found to be acting simultaneously. Conceptually, the mechanism of action can be thought of as the driving force for titer enhancement with in situ adsorption.

In situ adsorption acting to mitigate degradation or further metabolism

In some cases, the mechanism by which in situ adsorption is affecting natural product titers can be inferred from what is known about the stability of the molecule or about the fermentation in the absence of an adsorbent. Consider salinosporamide A, a natural product from the marine actinomycete, *Salinispora tropica,* which degrades rapidly in 0.05 M PBS, pH 6.5, with a half-life of 140 min [66]. The addition of 2 % XAD-7, 24 h post-inoculation, increased titers from 5.7 to 278 mg/l, indicating the resin is likely protecting the product from degradation. Furthermore, after reaching a peak concentration at about 96 h, titers of salinosporamide A in the presence of an adsorbent decreased from their maximum by 19 % over the next 48 h. In situ adsorption is therefore likely slowing the rate of degradation, but not preventing it entirely. Thus, when the rate of product degradation exceeds the rate of product synthesis and adsorption, titers of salinosporamide A decline. Whether adsorption of salinosporamide A is an equilibrium process and the degradation is occurring to the unbound fraction or whether degradation is occurring at a slower rate while the product is bound to the resin is unclear.

The presence of chemically unstable functional groups or structurally related metabolites from a common biosynthetic pathway may also highlight opportunities for using in situ adsorption to increase titers and/or alter metabolite profiles. Dynemicin A contains an unstable enediyne function that is readily metabolized to dynemicin H and a variety of other aromatized derivatives that lack the desired biological activity [38]. In the absence of an adsorbent, dynemicin H is the predominant species, comprising 65 % of all dynemicin production. The addition of an adsorbent resin (HP-20 or XAD-8) both increased the total production of dynemicins and shifted that production towards dynemicin A, with a concurrent 30-fold decrease in titers of the aromatized dynemicin H.

A similar situation exists in the production of paulomycin A and B by *Streptomyces paulus*. In the absence of an in situ adsorbent the dominant metabolites are the structurally related paulomenols, which lack the α-thiocyanatocrotonic acid group [46]. The presence of XAD-2 during the fermentation both shifts production in favor of paulomycin A and B and increases the combined production of paulomycins and paulomenols. The shift in the metabolite profile seen in both dynemicins and paulomycins suggests resins are acting to sequester an unstable intermediate. The fact that in both of these cases in situ

adsorption is also resulting in an increase in the total production of the biosynthetically related metabolites likely hints at the involvement of others mechanisms as well.

In situ adsorption acting to mitigate cytotoxicity or feedback inhibition

Both dynemicin A and the paulomycins are toxic when added exogenously to cultures of their respective producing microorganisms [38, 46]. This fact strongly suggests that increases in the titers of these metabolites with in situ adsorption are at least in part due to mitigating their autotoxicity. This mechanism has also been cited in the increases in titers seen with in situ adsorption of a number of other autotoxic natural products including, pristinamycin, kirromycin, teicoplanin, rubradirin and cercosporamide [5, 18, 41, 47, 63]. For example, the maximum titer of kirromycin in the absence of in situ adsorption and the minimum inhibitory concentration toward the producing organism during stationary phase are both about 350 mg/l [18].

It may be difficult, however, to distinguish between feedback inhibition of a specific biosynthetic pathway in secondary metabolism and broader metabolic effects that might be more appropriately classified as cytotoxicity. Sublethal concentrations of autotoxic molecules may well be having inhibitory effects that are not easily detected. Even looking for overt indicators of autotoxicity such as lower cell growth and loss of viability upon exogenous addition of the product can be difficult to interpret. This is because the sensitivity of many strains to the antibiotics they produce is known to decrease as the culture enters stationary phase when endogenous production typically begins [9, 12, 18, 47, 59]. Consider *Streptomyces achromogenes v. rubradiris* where the minimum inhibitory concentration of rubradirin is as low as 1 mg/l when added 24 h post-inoculation, but increases to 250 mg/l when the addition is made 72 h post-inoculation [47].

Looking for a decrease in antibiotic production in the absence of other metabolic changes, such as pH profiles and carbohydrate consumption rates, may be one means of distinguishing between effects of primary versus secondary metabolism. Exogenous addition of 1.2 g/l of cycloheximide to cultures of *Streptomyces griseus*, for example, completely shut down cycloheximide biosynthesis, while the glucose consumption rate remained nearly identical to cultures with no added cyclohexamide. This would seem to implicate feedback inhibition, as opposed to autotoxicity as a likely mechanism controlling cycloheximide biosynthesis [55].

In-situ adsorption impacting intracellular processes

An adsorbent can only bind natural products once they are outside the cell. So one may ask how titer improvements seen with in situ adsorption can be attributed to mitigating autotoxicity or feedback inhibition when the molecular targets of so many antibiotics (bacterial ribosome, intracellular enzymes, translation factors, RNA polymerase) and all the genetic, regulatory and enzymatic machinery for their biosynthesis reside inside the cell. To impact events inside the cell, in situ adsorption must be acting indirectly by altering intracellular concentrations of the inhibitory or autotoxic natural product. Consider the following material balance around a cell, keeping in mind that degradation in this context refers to that occurring inside the cell. Given that efflux pumps, such as the ATP binding cassette (ABC) transporters operating at the cell membrane, would be shielded from any direct contact with an adsorbent by the cell wall, the only possibility for in situ adsorption to impact intracellular concentrations is by acting as a natural product sink and thereby preventing or minimizing reuptake.

$$\text{Intracellular concentration} = \text{Biosynthesis} - \text{Degradation} - \text{Efflux} + \text{Reuptake}$$

Much of the literature on barriers to the uptake of natural products has focused on clinically relevant pathogens such as *Pseudomonas aeruginosa* and *Mycobacterium* where limited uptake of antibiotics is known to contribute to their antimicrobial resistance. The degree to which natural products, endogenously produced/exported or emanating from siblings, are reabsorbed by the producing strain itself has not been well characterized. Changes in cell permeability in antibiotic producing strains have been proposed as a self-defense mechanism to prevent resorption of toxic natural products [12, 59]. The experimental evidence for this, however, often does not satisfactorily distinguish between decreased permeability and up-regulation of efflux mechanisms, the genes for which are often under the same regulatory control as those of natural product biosynthesis. As has been previously noted, the reuptake of antibiotics by producing strains would represent a futile and costly cycle of ATP consumption given the efflux mechanisms involved in exporting the antibiotic from the cell [9]. Yet reuptake does occur, as many antibiotic producing strains are sensitive to the exogenous addition of the antibiotics they produce.

The principle barriers to resorption in bacteria are the cell membrane and, in the case of Gram-negative bacteria, the outer membrane. Many natural products of interest are antibiotics, which natural selection would have endowed with a capacity to penetrate these barriers in some significant portion of the microbial spectrum. Furthermore, the lack of a hydrophobic outer membrane in prolific natural product producers such as *Streptomyces* sp. presents a lower barrier to resorption for these and other Gram-positive strains. The exact mechanisms involved in

resorption of antibiotics by their producing strains have not been broadly studied and are likely to be strain dependent.

One of the few strains where this has been investigated is the streptomycin producer, *Streptomyces griseus*. Isotope labeling studies indicate reuptake of streptomycin into the cytoplasm of *S. griseus* does occur and is believed to be an active process involving a polyamine transport system [65]. In general, the cell wall in Gram-positive bacteria is not believed to constitute a significant chemical barrier. This, however, may not be entirely true of *S. griseus*, whose cell wall has been shown to contain some lipid components that may enhance its ability to serve as a permeability barrier [31]. In addition, streptomycin is known to reversibly bind to the cell surface of *S. griseus* by an ion-exchange mechanism [8]. A wide channel pore comprised of a 28-kDa protein has been identified in the cell wall of *S. griseus* [31]. This channel contains a binding site with specificity for streptomycin (kanamycin does not bind). The binding appears to be ionic in nature and may, in whole or in part, be the molecular basis for the observed binding of streptomycin to the cell surface. It is unclear if this channel protein is functioning as a transporter operating at the cell wall. If it is part of a transport mechanism then that presumes the cell wall is a significant permeability barrier. If it is not part of a transporter, then one must wonder what function it serves. It is interesting to consider that the cell wall in *S. griseus* may be preventing reuptake of streptomycin in much the same way as a synthetic adsorbent.

The barrier to resorption in Gram-negative bacteria is higher by virtue of its lipophilic outer membrane. Among the examples of in situ product adsorption cited in Tables 2 and 3, only six (18 %) are from Gram-negative bacteria. Doubtlessly, this is in part because fewer natural products of interest are produced by Gram-negative bacteria. Whether the increased barrier to reuptake makes in situ adsorption less effective in Gram-negative bacteria is unclear. Of the six Gram-negative bacteria where in situ adsorption was successfully employed, two are by mechanisms (preventing degradation in the fermentation medium and sequestering NH_4) that do not hinge on an external sink preventing reuptake of the product [39, 54]. Three others are by unknown mechanisms [28, 32, 43]. The soraphens and their producing strain, *S. cellulosum,* however, do provide one example where in situ adsorption appears to acting indirectly by decreasing intracellular concentrations in a Gram-negative bacteria. The evidence for this rests on the following: (1) soraphens are secreted in the fermentation medium, (2) their biosynthesis is regulated by feedback inhibition of the polyketide synthase as well as other downstream tailoring enzymes and (3) in situ adsorption has been shown to increase the total concentration of soraphens as well as shift the metabolite profile from Soraphen C to Soraphen A [19, 20].

With regard to the importance of efflux mechanisms, one recent review on the myriad of self-defense mechanisms employed by antibiotic producers concluded that the contribution of efflux to resistance, while difficult to measure, is pervasive [9]. This is supported by recent genomic sequence analysis suggesting significantly more genes of putative ABC transporters reside in prolific natural product producer like *Streptomyces* than in *Escherichia. coli, Bacillus. subtilis* and other microorganisms [48]. The importance of efflux is also supported by experiments where the introduction of multiple copies of known efflux genes into the corresponding producing strains have resulted in higher titers of cephalosporin, cercosporin and doxorubicin [44, 45]. While efflux mechanisms are usually regarded as a means of exporting endogenously produced antibiotics, their significance may be magnified if one considers that resorption of antibiotics by the producing strain may be underestimated. With all the regulatory machinery controlling natural product biosynthesis sensitive only to conditions inside the cell, resorption and efflux could be seen as flip sides of the same coin. Whether these two processes are of equal importance is up for debate, but the multi-fold increases in titer seen with in situ product adsorption would argue that perhaps they are. In this context, in situ product adsorption can be thought of as simply supplementing and/or working in concert with existing mechanisms (feedback inhibition, efflux pumps, adsorption of toxic molecules to the cell wall) that nature has honed for self-preservation.

Conclusions

In situ product adsorption using nonionic polymeric adsorbent resins has been shown to significantly increase titers and/or alter metabolite profiles in many natural product fermentations. The principle mechanisms responsible for increased product titers include one or more of the following processes; feedback inhibition, product instability and/or autotoxicity. The suspected presence of any of these three processes in a natural product fermentation makes it a good candidate for in situ product adsorption. *In-situ* adsorption has succeeded in increasing product titers over a broad range of product classes and producing strains, encompassing both Gram-positive and Gram-negative bacteria as well as fungi. As has been emphasized, in situ adsorption requires no additional investment in equipment and synergizes well with traditional strain improvement and fermentation medium development. The primary prerequisites may be a re-evaluation of sample extraction procedures and a fundamental understanding of some of the relevant variables.

Any resurgence of natural products in drug discovery pipelines must be accompanied by: (1) greater success at discovering novel natural products and (2) greater efficiency and lower costs associated with moving those candidates from the discovery phase into preclinical/clinical development. *In-situ* product adsorption can play a role in both of these areas. As mentioned, rapid low-cost DNA sequencing is revealing a potentially large untapped reservoir of natural products in the unexpressed and underexpressed biosynthetic pathways. Efforts are underway to access these reservoirs of chemical diversity. While there is no evidence to suggest in situ product adsorption is capable of activating unexpressed (cryptic) biosynthetic pathways it can facilitate discovery by increasing titers of underexpressed natural products and thereby aid in their detection (bioassay or spectrographic) and isolation. Considering the mechanisms involved in titer enhancement, the use of in situ product adsorption in generating natural product libraries would tend to select for compounds that may have potentially desirable or intriguing attributes, including (1) cell permeability, (2) cytotoxicity, (3) the presence of other biological activities for which feedback inhibition mechanisms have evolved and (4) biosynthetic intermediates that may be structurally unique (patentable) and/or contain reactive handles useful for semi-synthesis.

Transitioning a lead molecule into preclinical/clinical development has many risks and hurdles; one of which is generating sufficient quantities of the drug candidate to sustain a robust preclinical/clinical development program. While this has been perceived as being particularly challenging for natural products, there are significant costs and risks in scaling up lead molecules derived from combinatorial and fragment-based libraries as well. For natural products to compete favorably with synthetic routes of drug development, every available means of expediting natural product fermentation development should be considered, including in situ product adsorption strategies.

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