Biosafety in Cell Factory

INTRODUCTION

In the past 100 years, the rapid development of traditional chemical industry has greatly enhanced the quality of life of people. However, the pollution and destruction brought by the chemical industry are also devastating our planet. The emergence of synthetic biology helps to provide us with a new solution; we can introduce new chemical reactions into biological cells, thus getting high-quality chemical products in a greener way.

Whether it is a breakthrough in new technology or the pressure from the reality of environment, more and more people are coming to the belief that in the near future, green biological cell factory will have to replace the existing chemical industry.

In terms of environmental safety, cell factories have a natural advantage over traditional chemical ones, but they are also facing new problems. The potential biosecurity of the cell factory, especially the transgenic safety problem, can bring to the environment more irreversible safety risks.

Therefore, the following problems deserves our careful consideration.

1. Do the existing laws and regulations touch on the specific provisions regarding the biosafety of biological manufacturing factories (biological pharmaceutical factories, chemical factories, etc.), especially the prevention of genetic pollution? What are the differences in the standards of several major countries or regions?
2. Have the previous iGEM teams conducted corresponding researches on this aspect?
3. How are the existing biotech companies taking action on biogenetic safety? What is the current solution? Are there any drawbacks?
4. As for the future cell factories, is there a better safety measure to boost the protection against biological genetic safety problems? And should the government have more specific mandatory requirements to help control the biosafety risks of the future cell factories?

In view of the above genetic problems of cell factories, NPU-China and SCUT-FSE-CHINA together made the investigation from the following aspects:

1.Corresponding laws and regulations regarding biosafety worldwide.
2.The main technical solution of the sewage treatment in chemical plants.
3.The level of understanding of biosafety concerning cell factories of the previous iGEM teams.

On the basis of our research, we have also proposed solutions that we consider viable, and we will organize these results into reports so that we will be able to deepen our understanding of the future biosafety of cell factories and hope to provide a certain reference to the future cell factory designers or supervisors

CONTEXT

Biosafety Regulation

We searched for the current laws and regulations about GMOs in several different countries or regions, especially the factory emis-sions' parts. The following will be our findings and expectations.

EUROPEAN UNION

Refer to On the contained use of genetically modified micro-organisms (GMOs) (90/219/EEC, 98/81/EC) and On the deliberate re-lease into the environment of genetically modified micro-organisms (90/220/EEC，97/35/EC) ,combine with the information offered by Groningen University (Netherland), we found out that laws and regula-tions in EU mainly concerned about the possible or potential risks of genetic modification techniques during products processing as well as the safety evaluation of the final GMO products, so there have very strict evaluation system in GMOs biosafety.

However, they don't have very clear rules about the waste of GMOs products. According to Part B, Article 5 from On the deliberate release into the environment of genetically modified micro-organisms (90/220/EEC，97/35/EC), the deliberate release of GMOs for any other purpose than for placing on the market should carry out assessment of the risk to environment, but the committee did not mentioned the later procedure. And we also learned that, the EU GMOs control system do not work efficiently, because of some differences and contradictions in some of their member countries.

USA

The GMOs management in the USA is operated by USDA, EPA and FDA. The USA has the longest history in GMOs management as well as the most completed regulations of it. The laws and regulations on bi-osafety and genetically modified food management in the United States are relatively sound, and the departments are relatively independent, with clear division of responsibilities, clear powers and responsibilities, harmonization and high efficiency. As the United States on the man-agement of genetically modified food to adopt a relatively liberal policy, the United States of genetically modified crops and genetically modified food development is very fast. And the management of each department depends on the end use of the GMO product. A product may involve the management of multiple departments.

However, their management system still has several dissatisfying prob-lems. Firstly, it doesn't have identification of genetically modified food or other products. Secondly, like the other two region above, they didn't include detailed restrictions about environmental release of GMOs.

CHINA

The rules of the deliberate release of GMOs in China are restricted by Regulations on Administration of Agricultural Genetically Modified Organisms Safety. The permission of environmental GMOs release is given by Ministry of Agriculture of the People's Republic of China. But Chinese government didn't recommend any technique in processing the GMOs wastes, and as the regulation mainly focus on biosafety evaluation, the environmental release part is almost blank in this regulation.

表1　现有企业水污染物排放限值

单位为mg/L(pH值、色度、粪大肠菌群数除外)

序号	污染物项目	排放限值	监控位置
1	pH 值	6~9	企业废水总排放口
2	色度　（稀释倍数）	80	
3	悬浮物	70	
4	五日生化需氧量（BOD_5）	30	
5	化学需氧量（CODcr）	100	
6	动植物油	10	
7	挥发酚	0.5	
8	氨氮（以N计）	15	
9	总 氮	50	
10	总 磷	1.0	
11	甲 醛	2.0	
12	乙 腈	3.0	
13	总余氯（以 Cl 计）	0.5	
14	粪大肠菌群数[1]　（MPN/L）	500	
15	总有机碳	30	
16	急性毒性（$HgCl_2$ 毒性当量）	0.07	

注1）：消毒指示微生物指标。

From the bio-engineering pharmaceutical industry water pollutant dis-charge standards (GB 21907-2008)(see left), we can initially build some ideas on cell factory to build sewage treatment methods , basing on chemical treatment technology and increasing the attention and treatment of gene flow. Thus conducting initiatives and planning for ge-netic contamination,as well as Bio Safety.

—Treatment of Sewage ——

There are some sewage treatment methods of the most chemical factories currently:

1.Physical method (including filtration, gravity precipitation and air floatation, etc.);
2.Chemical method (chemicalcoagulation,chemical oxidation,electrochemical oxidation, etc.);
3.Biochemical method (activated sludge method, SBR method, contact oxidation process, ascending anaerobic sludge bed, etc.);
4.Physical chemistry method (adsorption, extraction, film suction, etc.).

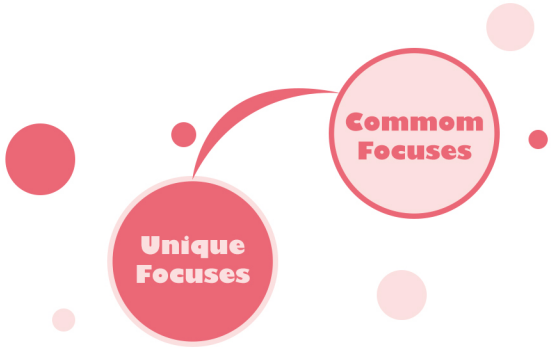
There are several new chemical pretreatment methods that can effective-ly reduce the COD content of sewage:

1.Catalytic microelectrolysis technology: the use of micro-electrolysis equipment filled with micro-electrolysis filler to produce "original bat-tery" effect on the treatment of wastewater to improve the biodegradabil-ity of sewage.
2.New catalytic micro-electrolysis filler technology: by the multi-metal al-loy fusion catalyst and the use of high-temperature microporous activa-tion technology is produced, is a new type of no-type micro-electrolytic packing.
3.Heterogeneous catalytic oxidation technology: the use of hydroxyl radi-cals as the core of the strong oxidants, fast, no selectivity, complete oxi-dation of the environment in a variety of organic pollutants.
Combined with membrane separation technology to achieve better re-tention and interception.

How the Previous iGEM Teams focus on Biosafety?

Here we surveyed all the undergraduate that obtained gold medals in 2016, and sorted out a list of biosafety concerns of different teams. We found that many teams share something in common regarding biosafety. Of course there are teams with their unique practice.

So we sorted out the following chart by two parts, which are Common Focus and Unique Focus:



Common Focuses

Common Focuses	Lab Safety	Project Safety	Safety Training	Links of Relevant Laws	Transportation Safety
Team number	23	20	10	8	15
Remark	Differs from each university	Distinct descriptions accordingly	A title followed by detailed contents	Differs from each university	

Unique Focuses

1. RWTH Aachen conducts toxicology assessment and applies numerous professional formulas.
2. Harvard and SYSU both employ questionnaires. Harvard presents all 5 questions on the website while SYSU attaches the relevant link.
3. During gel extraction, Manchester utilizes SYBR Safe as an alternative, thus reducing the amount of EtBr used and lower the risk of mutagenesis.
4. INSA-Lyon and Newcastle list all the toxic reagents.
5. Newcastle states the nontoxicity of the protein involved and its high productivity in L-arabinose, which is strictly required.
6. NUDT acknowledges the nontoxicity of the protein involved.
7. NUS expounds the glory of their laboratory in the very beginning of the biosafety.
8. Peking University touches on the prevention of escape and leakage of the engineered bacteria from the very source.
9. SCAU firstly introduces the significance and necessity of biosafety.
10. Based on rice, the related regulations and laws of main countries are included in their biosafety. And describes the experiment process in a professional way.
11. Dundee University make the risk assessment of their chemicals and mentions the security items during experiments. Also, innocuous E. Coli MG1655 is adopted.
12. FAFU uses the safe Chlamydomonas reinhardtii, whose recombination suppression sequence on sex chromosomes can impede the recombination between chromosomes. A special pair of primers was designed to detect the Safety Tag which owns no homology to the other DNA fragments.

CONCLUTION

About regulations:

Through the global biosafety regulations study, we find out that envi-ronmental release of GMOs is an underestimated session in the countries or regions that we searched, because there are almost no detailed re-strictions about it. All the regulations come to a stereotype that the emis-sions program depends on local environment situation and the factory itself. However, it's an unavoidable reason that cause danger to the raw ecological environment, which will directly influence the biological di-versity in original natural ecosystem. Measures should be taken to fill up the current blank.

About treatment:

Although genetically modified organisms, including factories and ani-mals and microorganisms, all have gene flow and genetic contamina-tion, only a few crops are getting in commercial production currently. So at present, there is much discussion about the risk of GM crops. As for the industrial production of bulk chemicals by microbiological cells, there is a lack of uniform standards and norms in the public sight.

So we can focus on the specific content of the operation of the laborato-ry to explore the corresponding waste disposal methods. For example, when we transform engineering bacteria, the application of genetic en-gineering methods to optimize the target bacterial strain's metabolic pathway, and foreign genes will be imported into the wild type; for the convenience of screening and other operations, resistance genes are of-ten transferred into the strain, and so on.

About Previous iGEM Teams:

According to our investigation, we found out that many teams fixed their attention on something in common. Many a team concentrate on the lab safety, safety training and transportation safety, etc. Additionally, we also noticed that no teams, till now, focus on the biosafety of the future cell factory. Hence, we are confident about innovation of our Human Practice for its unique and innovative ideas. We expanded upon genetical leakage, genetical contamination and some other aspects related. We have explored a more tangible and original prospective to show our concern about biosafety.

The term "gene flow" comes from a English word “flow”, which mainly refers to the exchange of genes between individuals or groups due to hybridization. In the literature of biosafety, it mainly refers to the flow between trans-genic organisms and their relatives. Gene flow has two ways to achieve. The first one is in the natural state to carry transgenic pollen through the wind or insect propagation. Once in the environment to find the recipient and the formation of fertile or infertile hybrid offspring, the transgenic process to the environment will be completed. In the transgenic pro-cess, of course, genetically modified organisms can also be used as pollen receptors to carry transgenes of the offspring. Secondly, transgenic organisms, especially transgenic facto-ries after harvest, the seeds may be scattered in the envi-ronment to form the self-propagating factories (“Volun-teers”), After flowering, it can be hybridized with the wild near edge, and the self-seeding plant with the selective ad-vantage may become a weed.

These two processes are also known as transgenic es-cape in foreign literature. The first form, spread of pollen, is also known as Pollen drift.

Gene flow & Gene contamination

Gene contamination is used to refer to foreign genes that transgenic organisms are transferred and integrated in-to the genome of other organisms in some way, so that other organisms, especially plant seeds or products, are mixed with transgenic ingredients, resulting in a mixture or pollution of natural gene banks. In general, from the relationship be-tween gene flow and gene contamination, gene flow is the cause,and genetic contamination is consequence. In theo-ry, as long as blocking gene flow from the source can we prevent genetic pollution.

SUGGESTION

Regulation suggestion:

1. Related department should put more emphasis on environment risk assessment and safety monitoring as well as further the technical research work on the envi-ronmental emissions of GMOs, which including toxicity testing, allergies testing, resistance analysis, animal experiment or assessment of GMOs emissions for human health, agricultural ecological environment potential hazards.
2. The current management needs to be consummated by filling the management regulations and procedures up with clear restrictions, detailed methodology, in order to make the GMOs emissions friendlier to the environment.
3. During the early stage of synthetic biology development, the potential problems of it should be taken into consideration in the first place, for the uncertainty of GMOs can be easily influenced by undesirable factors. Taking actions to prevent the negative situation will allow the synchronization between risk evaluation and technique development and avoid environmental risk evaluation lagging behind.

Treatment Suggestion:

We can focus on the specific content of the operation of the laboratory to explore the corresponding waste disposal methods. For example, when we transform engineer-ing bacteria, the application of genetic engineering methods to optimize the target bacterial strain's metabolic pathway, and foreign genes will be imported into the wild type; for the convenience of screening and other operations, resistance genes are often transferred into the strain, and so on.

The left figure shows the distribution of different types of resistance genes and antibiotics in different soil environment media in China.

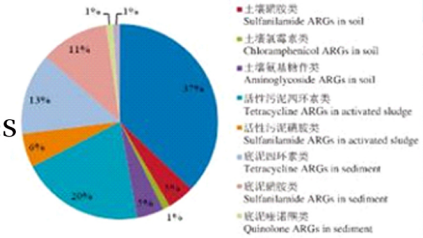


图1 我国不同土壤环境介质中不同种类ARGs的研究比例分布.
Fig. 1 Research distribution for different ARGs in the soil environment of China.

表3 不同处理工艺对ARGs的去除效果
Table 3 ARGs removal efficiency of different treatment processes

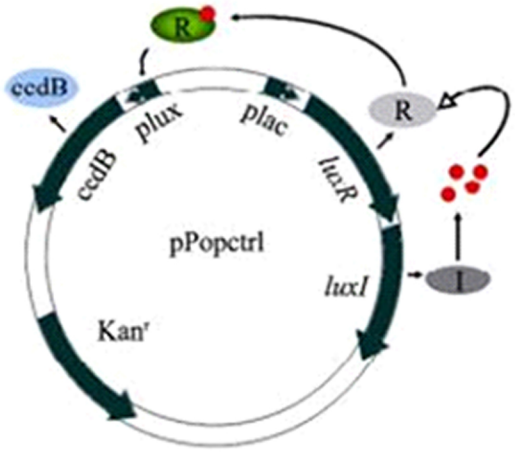
分类 Method	处理工艺 Treatment Processe	ARGs去除效果 ARGs removal efficiency
物理法 Physical	初次沉淀、过滤 primary sedimentation and filtration	*tetA*浓度减少了0.03-0.4 log单位，*tetB*减少了0.03-0.7 log单位[94] *tetA* and *tetB* reduced by 0.03-0.4 and 0.03-0.7 log unit, respectively [94]
	紫外消毒 Ultraviolet disinfection	甲氧西林（*mecA*）抗性基因去除量为1个数量级，万古霉素（*vanA*）抗性基因无明显去除[95] *mecA* reduced by 1 order of magnitude and no obvious removal to *vanA* [95]
	石灰稳定法 Lime stabilization	*sul* 1 去除效果约1 log单位，*tetO*去除效果约0.4 log单位[95] *sul* 1 and *tetO* reduced by 1 and 0.4 log unit, respectively [95]
化学法 Chemistry	臭氧消毒 Ozone disinfection	金霉素、林可霉素、磺胺甲唑啶和四环素抗性细菌的去除效果为3.3-3.9 log单位[96] Aureomycin, lincomycin, sulfamethazine and tetracycline resistant bacteria reduced by 3.3-3.9 log unit [96]
	氯消毒 Chlorine disinfection	金霉素、林可霉素、磺胺甲唑啶和四环素抗性细菌的去除效果为2.2-3.4 log单位[96] Aureomycin, lincomycin, sulfamethazine and tetracycline resistant bacteria reduced by 2.2-3.4 log unit[96]
生物法 Biology	活性污泥 Activated sludge process,	*tetA*浓度减少了0.4-1.5 log单位，*tetB*减少了0.5-1.6 log单位[94] *tetA* and *tetB* reduced by 0.4-1.5 and 0.5-1.6 log unit, respectively [94]
	滴滤池 Trickling filter	*tetA*浓度减少了0.6-1.5 log单位，*tetB*减少了0.6-1.3 log单位[94] *tetA* and *tetB* reduced by 0.6-1.5 and 0.6-1.3 log unit, respectively [94]
	MBR Membrane Bio-Reactor	*tetW*、*tetO*去除效果达2.57-7.06 log单位[97] *tetW* and *tetO* reduced by 2.57-7.06 log unit [97]
	厌氧消化 Anaerobic digestion	*sul* 1 去除效果约0.5 log单位，*tetO*去除效果约1 log单位[95] *sul* 1 and *tetO* reduced by 0.5 and 1 log unit, respectively[95]
	人工湿地 Constructed wetlands	*tetW*、*tetM*和*tetO*减少了50%-90% [98] *tetW*, *tetM*, *tetO* reduced by 50-90% [98]

The spread of the resistance gene in the environment is related to the level of gene transfer and microbial community structure. The natural degradation process of antibiotics and resistance genes in the environment is affected by factors such as substrate type, light, temperature and microbial population, which is an important factor affecting its degradation. In the artificial processing system, UV disinfection and biodegradation (such as activated sludge method) have a good effect on the removal of antibiotics and their resistance genes , but not all of those methods effective.

Thus, we can treat sewage from two aspects of genetic level: First, insert a suicide system into the target strain to cause the strain to apoptosis in a non-productive state, making the strain in the non-production state of self-apoptosis; Second, on this basis we can use

some chemical methods for degradation.

And the population-based suicide gene loop can give the host bacteria the ability to initiate suicide at a certain density. The periodic oscillations of the strain density can be formed under continuous culture conditions. Such as You in United States Duke University has established a suicide bacteria model and the oscillation of genet-ic components, using the elements of group induction and AHL-LuxR ,a pair of self-inducer - transcription factor molecules, to build a more robust suicide gene loop (see below).



Above-mentioned is our current level of treatment that can be achieved in the laboratory or in the factories' application. We are committed to formulating reasonable ideas for the industrialization of synthetic biology to promote the process of industrialization. We sincerely hope that you will be able to make your valuable suggestions or comments on the methods of sewage treatments, as well as the Bio Safety aspect, thank you so much !