

PDF-Export

Topic:

Bulk 1

Paper-ID 29836

Paper title (Hemi)cellulolytic enzymes production from *Bosea* sp. FBZP-16 on different lignocellulosic substrates under submerged fermentation in shake flask cultures

Presentation format Oral presentation

Date of submission 26.01.2017 11:58 Uhr

Authors

- Aicha Asma Houfani (*a.a.houfani@gmail.com*) (Presenter)
- Nico Anders (*Nico.Anders@avt.rwth-aachen.de*)
- Jochen Büchs (*jochen.buechs@avt.rwth-aachen.de*)
- Tomáš Větrovský (*kostelecke.uzeniny@seznam.cz*)
- Petr Baldrian (*baldrian@biomed.cas.cz*)
- Said Benallaoua (*sbenal@hotmail.com*)

Content

Cellulose and hemicellulose represent the most abundant renewable resources on earth. However, the competition of these renewable resources is omnipresent, thus, agricultural, forestry, industrial and urban lignocellulosic wastes are preferable.

In recent years, increasing attention has been devoted to the production of bioethanol from low cost lignocelluloses. However, the majority of the commercial enzymes for biomass degradation are of fungal origin due to their higher activity. Biomass-degrading enzymes originating from bacteria, however, can be a viable alternative to existing fungal systems [1]. Indeed, enzymes from bacteria with the combination of using agro-industrial residues, can reduce significantly the costs in the production of fuels and chemicals derived from plant biomass.

The aim of this study was the evaluation of isolating lignocellulosic degrading enzymes out of a local Algerian bacterial strain by using different lignocellulosic raw materials such as orange peel, barely awn and spruce wood with minimal medium (0.7% KH_2PO_4 , 0.2% K_2HPO_4 , 0.05% $\text{MgSO}_4 \times 7\text{H}_2\text{O}$, 0.1% $(\text{NH}_4)_2\text{SO}_4$, 0.06% yeast extract).

The chosen strain (GenBank accession numbers) *Bosea* sp. FBZP-16 (KT868785) was isolated from a forest soil and had been shown in previous experiments to be involved in cellulose and hemicellulose decomposition using microbiological, biochemical, and next-generation sequencing biochemical screening steps [2]. The tested lignocellulosic raw materials were previously analysed using high performance anion exchange chromatography coupled to pulsed amperometric detection [3]. In this study, it turned out that *Bosea* sp. FBZP-16 presented the ability to produce either cellulases or xylanases, or both enzymes on different lignocellulosic biomass and thus can be potential candidate for its application in the production of fermentable sugars.

References:

1. Berlin, A. (2013). No Barriers to Cellulose Breakdown, *Science* 342, 1454.
2. Houfani A. A., Větrovský T., Baldrian P., Benallaoua S. (2017). Efficient screening of potential cellulases and hemicellulases produced by *Bosea* sp. FBZP-16 using the combination of enzyme assays and genome analysis. *World Journal of Microbiology and Biotechnology* 33 (29): 1-14.
3. Anders, N., Humann, H., Langhans B. and Spieß, A. C., (2015) Simultaneous determination of acid-soluble biomass-derived compounds using high performance anion exchange chromatography coupled with pulsed amperometric detection. *Analytical Methods*, 7, 7866-7873.

Paper-ID 70756
Paper title Effect of Lignin Scavengers on Cellulose Saccharification
Presentation format Poster
Date of submission 20.02.2017 11:27 Uhr
Authors
- Priscilla Vergara (*privergara@gmail.com*) (Presenter)
- José María Carbajo (*chema@inia.es*)
- Miguel Ladero (*mladero@quim.ucm.es*)
- Félix García-Ochoa (*fgochoa@ucm.es*)
- Juan Carlos Villar (*villar@inia.es*)

Content

Introduction

Lignocellulosic biomass from agriculture and forestry is the most abundant bioresource to be employed as feedstock for biorefineries. However, because of the complex structure of the lignocellulosic biomass, a pretreatment enhancing the susceptibility of the cellulose to enzymatic hydrolysis is required for conversion to biofuels or platform chemicals. During the pretreatment lignocellulose-derived by-products are produced, which have an inhibitory effect on the downstream biochemical processes. The use of additives such as surfactants or non-catalytic proteins has been described in the literature to enhance cellulose hydrolysis by enzymes. The objective of this work is to determine the effect of the use of different additives on the performance of the enzymatic hydrolysis, based on the idea that the conversion of cellulose to glucose would be improved by increasing the enzyme amount available for the hydrolysis.

Experimental

Wheat straw was fractionated with an ethanol-water mixture with 1% of sulfuric acid (dry wheat straw). Two pretreatment conditions were checked; the first pretreatment was performed with low energy to cause low delignification and the second with high energy to obtain a highly delignified material. The pretreated material was filtered and the solid fraction washed. Unpretreated and pretreated materials were enzymatically hydrolysed to evaluate cellulose digestibility. The conditions were: 0.05 M citrate buffer (pH 4.8), 50°C, 150 rpm for 72 h, 10% consistency and an excess of the enzyme mixture Biogazyme 2X. The reaction mixtures were supplemented with one of the five additives (BSA, casein, Tween 80, powder skimmed milk and PEG 6000) studied. These additives were added at three different concentrations. Prior to enzyme addition to start hydrolysis, the mixtures were incubated for 24 h (50 °C, 500 rpm) to allow the additives disperse uniformly in the buffer in order to interact with the substrate. All runs of hydrolysis were performed in duplicate. Glucose was determined using a HPLC technique at 24, 48 and 72 hours and de yield of the hydrolysis calculated.

Results and discussion

When the low delignificated material (47.7%) was employed, after 48 hours of enzymatic hydrolysis, the employment of additives yields best results compared to the control run. The best additives were BSA (0.1 g/g) and Casein (0.5 g/g), producing a yield increase of 12.3 % and 12.2 %, respectively.

On the other hand, for the material with a high degree of delignification (73.1 %), also after 48 h of enzymatic hydrolysis, the best results were obtained using Tween 80 (10/1000 v/v) and BSA (0.1 g/g), improving the yield by 6.4% and 5.8%, respectively. It should be noted that this lower improvements in yields when using additives in this material may be due to the lower lignin content.

Paper-ID 95696

Paper title Effect of γ -valerolactone on the hydrothermal conversion of biomass under acid-free conditions

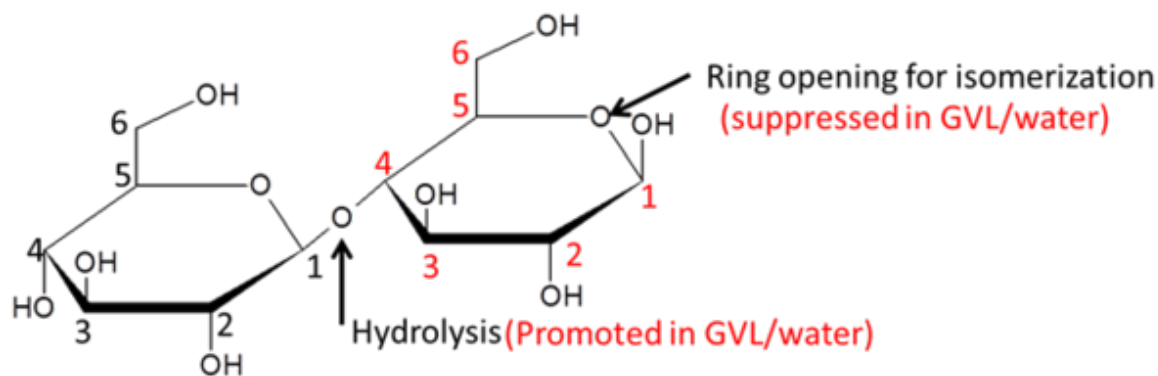
Presentation format Oral presentation

Date of submission 31.03.2017 12:31 Uhr

Authors - Bing Song (*bing.song@student.curtin.edu.au*) (Presenter)
 - Yun Yu (*Yun.Yu@curtin.edu.au*)
 - Hongwei Wu (*h.wu@curtin.edu.au*)

Content

Hydrothermal treatment of biomass is a key process for converting biomass to biofuels and value-added biochemical. A biomass-derived chemical, γ -valerolactone (GVL), is considered to be a green solvent for biomass treatment and further conversion. This study provides new insights into the solvent effect of GVL on the hydrothermal conversion of biomass under acid-free conditions with glucose, cellobiose, and cellulose as model molecules. It was found that GVL addition even at a small concentration suppresses the isomerization reactions, which dominate the cellulose conversion in hot-compressed water. For glucose, the primary selectivity of fructose (isomer of glucose) can be significantly suppressed from 80% in pure water to 10% in 50/50 GVL/water. For cellobiose, the primary selectivity of glucosyl-fructose (isomer of cellobiose) can be suppressed even when GVL is at 0.03 wt%, and an increase of GVL to 25 wt% can eliminate the isomerization of cellobiose. Meanwhile, the maximal selectivity of glucose is strongly increased to 80% compared with that in pure water at 30%. Further study on cellulose suggests that the peaks for isomers on a high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) are eliminated and the glucose recovery after post-hydrolysis is increased from 80% in water to 92% in 10/90 GVL/water. The results demonstrate that GVL/water mixture solvent can suppress the isomerization of cellulose and glucose oligomers under acid-free conditions.



Paper-ID 83266
Paper title Lignocellulosic Biomass Pre-treatment: Diluted Acid and Solvent Delignification
Presentation format Oral presentation
Date of submission 28.02.2017 11:46 Uhr
Authors
- Priscilla Vergara (*privergara@gmail.com*) (Presenter)
- Miguel Ladero (*mladero@ucm.es*)
- Felix García-Ochoa (*fgochoa@ucm.es*)
- Juan Carlos Villar (*villar@inia.es*)

Content

In a biorefinery scheme, the biomass supply is one of the main points that must be guaranteed. Multiple feedstock permits a bigger production and takes advantage of the scale economy. Lignocellulosic biorefineries use plants residues or crops to obtain sugars and lignin separately. One requisite is that feedstock must not compete with foodstuff production, what made agroforestry residues, as cereal straw or corn stalks, interesting raw materials. Marginal lands have limited potential to produce industrial crops in rural areas. Cardoon is an interesting biomass for marginal lands since it has a high production potential and is a multifunctional crop with different applications.

Moreover, the Lignocellulosic materials need a deconstruction that permits their further hydrolysis to sugars and lignin. The lignin-hemicelluloses matrix and the cellulose crystalline regions are barriers that block the enzymatic hydrolysis of polysaccharides. Among the proposed pretreatments, diluted acid hydrolysis and solvent delignification have retained interest because there are two promising methods for lignocellulose fractionation. The aim of this work is the study of the fractionation of three feedstocks (cereal straw, corn stalks and cardoon stalks) by the pretreatments of diluted acid hydrolysis and solvent delignification. The results were evaluated with the criteria of maximizing glucose production at a moderate energy requirement.

Wheat straw, cardoon and corn stalks were fractionated with an ethanol-water mixture (2/1 v/v) adding a 1% of sulphuric acid or alternatively, with a diluted sulphuric acid solution. The experiments were carried out in a 6 L reactor provided with liquor recirculation, temperature control and sampling. The variables to be evaluated were temperature and time. Solid fraction were removed at the end of the experiments, washed, weighed and enzymatic hydrolyzed with a mixture of commercial cellulases. The sugars in the liquefied fraction was analysed by HPLC.

For the evaluated materials it was observed that the yield of the solid fraction is mainly dependent on the fractionation temperature and ranges from 40% (170°C) to 85% (130 °C) were obtained. Also, for a similar energy input, the ethanol-water liquor is more selective to remove lignin and the acid hydrolysis dissolves most of the xylans, while ethanol-water treatments preserve most of them. The pretreated materials obtained using the ethanol-water mixture showed a better performance compared to those obtained with dilute acid. For wheat straw the maximum yield of enzymatic hydrolysis obtained is 80% while for Cardoon stalks only 54% was achieved. For similar energies provided, the pretreated materials obtained using the ethanol-water mixture showed a better performance compared to those obtained with dilute acid. For wheat straw the maximum yield of enzymatic hydrolysis obtained is 80% while for Cardoon stalks only 54% was achieved. Corn stalks showed an intermediate behavior.

Paper-ID 74446
Paper title Optimization of acid sulfite pretreatment in the enzymatic hydrolysis of *Cytisus striatus*
Presentation format Oral presentation
Date of submission 25.02.2017 13:46 Uhr
Authors - Álvaro Vaz (*avaz@ubi.pt*) (Presenter)
- Tânia Gomes (*taniarpgomes@gmail.com*)
- Rogério Simões (*rms@ubi.pt*)

Content

Ethanol production from lignocellulosic material includes three major steps: biomass pretreatment, which fragments the lignocellulosic matrix to facilitate the enzymes access to the substrate; hydrolysis, where the polysaccharides are converted into fermentable sugars (e.g. glucose and xylose) [1]; and finally, fermentation that produces ethanol or other biologically based chemicals (e.g. lactic acid, succinic acid) [2]. The aim of the present work was to study the effect of some operative variables of the pretreatment stage, namely sodium hydrogen sulfite and sulfuric acid loadings, temperature and time, on the release of sugars in the enzymatic hydrolysis of *Cytisus striatus*, performed applying a Novozymes® cocktail, with fixed charges and operating conditions. Wood branches were chipped and submitted to different reaction conditions, with a central composite experimental design 2⁴+star, exploring the following variables: sulfuric acid charge (0-3%, on wood), sodium bisulfite charge (0-4 %, on wood), maximum temperature (150-190°C) and time at maximum temperature (0-30 minutes). After pretreatment, the acid hydrolysates were recovered and the solid residues were mechanically disintegrated and thereafter subjected to enzymatic hydrolysis with an enzymatic cocktail from Novozymes®. Sugars and by-products released in the sulfite pretreatment and enzymatic treatment hydrolysates were analyzed by HPLC. The percentage of material released in the acid hydrolysates was between 7 and 29.1%. Temperature and sulfuric acid load were the most important tested variables. The inhibition products represented less than 0.9% of the initial wood mass, even for the most severe reaction conditions. Enzymatic hydrolysis of polysaccharides on solid residues resulted in conversions from 6.0 to 68.9%, depending on the reaction conditions used in biomass pretreatment with sulfite and sulfuric acid. The rate of sugars release proved to be high at the beginning, gradually decreasing with contact time. The experimental data analysis using Statgraphics®Plus5 enabled us to obtain correlation expressions and conclude that all study variables influence this phase of the process, mainly temperature and sulfuric acid load. For a given sulfite load, more acidic conditions led to higher sugar release and greater fragmentation of the material, but also higher production of degradation products; moderate sodium bisulfite (1%) and sulfuric acid (2.25%) loads released practically all hemicelluloses in the raw material.

1. Vera L. D. Costa, Tânia P. Gomes & Rogério M. S. Simões (2016) Effect of Acid Sulphite Pretreatment on Enzymatic Hydrolysis of Eucalypt, Broom, and Pine, 2016. *Journal of Wood Chemistry and Technology*, 36(1): p. 63-75.
2. Gil N, Domingues FC, Amaral ME, Duarte AP. Optimization of dilute acid pretreatment of *Cytisus striatus* and *Cistus ladanifer* for bioethanol production, 2012. *Journal of Biobased Materials and Bioenergy*, 6 (3): p. 292-298.

Paper-ID 89656

Paper title Reduction of cellulose crystallinity by heterogeneous pretreatment with a non-dissolving ionic liquid

Presentation format Oral presentation

Date of submission 28.02.2017 21:03 Uhr

Authors

- Héctor Rodríguez (*hector.rodriguez@usc.es*) (Presenter)
- Ashley J. Holding (*ashley.holding@helsinki.fi*)
- María C. Castro (*maria.castro@usc.es*)
- Daniel Rico del Cerro (*daniel.ricodelcerro@helsinki.fi*)
- Ilkka Kilpeläinen (*ilkka.kilpelainen@helsinki.fi*)
- Alistair W. T. King (*alistair.king@helsinki.fi*)

Content

Cellulose is an abundant, geographically-distributed, and biorenewable resource with the potential to be used as the raw material for the sustainable production of numerous chemical products and commodity materials. One of the major problems for its optimal exploitation is that cellulose is present in Nature with a high proportion of the 'cellulose I' crystalline polymorphs, within recalcitrant lignocellulosic matrices (e.g. wood). The conventional methods currently in use for obtaining cellulose are generally based on the degradation of the rest of the lignocellulosic matrix. These methods use corrosive textile-era chemicals and can have a severe environmental footprint for the older less regulated sites, especially in the developing world. These processes preserve the native crystallinity of the cellulose, as cellulose I, which limits its chemical reactivity to traditional heterogeneous processing. To increase its reactivity and processability, cellulose can be subjected to a mercerisation-type process which transforms cellulose I into the more thermodynamically stable and more accessible crystalline form, denominated as 'cellulose II'. This is a heterogeneous procedure as cellulose is not dissolved during the procedure. However, reduction in cellulose crystallinity, to yield amorphous cellulose would be much more desirable as kinetics of chemical reactivity should increase well beyond that for cellulose II.

It is known that some ionic liquids are able to dissolve cellulose under mild conditions. During regeneration, cellulose II is typically obtained via addition of a protic antisolvent. The recovery of the cellulose and ionic liquid in an efficient manner, under such conditions, remains a critical issue from a process perspective.

In this work, we have discovered that some ionic liquids without the ability to dissolve cellulose in appreciable amounts are however capable of reducing the degree of crystallinity of cellulose, through a heterogeneous pretreatment of either a pure technical cellulose or even directly wood chips in which it is contained. An analysis of the improved reactivity of the pretreated cellulose has been carried out, using some typical methods for transformation of cellulose. As such, we would like to present these results as we believe that this could yield access to new cellulose-based chemicals, through lower cost heterogeneous processing, or possibly even enhance biofuels production from woody biomass.

Paper-ID 73576
Paper title Solvent pretreatment of *Cynara cardunculus*: Influence of operational conditions
Presentation format Poster
Date of submission 24.02.2017 14:58 Uhr
Authors - PRISCILLA VERGARA (*privergara@gmail.com*) (Presenter)
- MIGUEL LADERO (*mladero@quim.ucm.es*)
- FÉLIX GARCÍA-OCHOA (*fgochoa@ucm.es*)
- JUAN CARLOS VILLAR (*villar@inia.es*)

Content

Agroforestry crops are renewable and ubiquitous lignocellulosic materials, with high polysaccharides content and a potential source of sugars to convert into bio-based products. Some of them, as cardoon, has an additional interest due to the possibility of been cultivated in marginal land.

In lignocellulosic materials, the enzymatic hydrolysis of cellulose into glucose is inhibited by the hemicelluloses-lignin matrix and by the crystalline regions of the cellulose. Therefore, a pretreatment is necessary to make the cellulose accessible to the enzymes. The ethanol-water fractionation of lignocellulose is an effective pretreatment to produce sugars and lignin within a biorefinery scheme. The objective of this study was to optimize the ethanol-water fractionation of *Cynara cardunculus* (cardoon) stems by using the Taguchi method. The effect of the fractionation parameters on the recovery of sugars in the solid residue will be optimized.

In this work is used a L9 orthogonal array scheme which nine experiments to complete the optimization of pretreatment process. The parameters considered were temperature (150, 170, 190°C), fractionation time (60, 90, 120 min.), liquor-cardoon ratio (20, 25, 30 L/Kg) and ethanol percentage in liquor (0.25, 0.33, 50%). For each parameter, three levels were tested to minimize the signal to noise ratio (larger-the-better). The experiments were carried out randomly to avoid any kind of bias.

Cardoon stalks (100 g) was fractionated with an ethanol-water mixture and 1% of sulphuric acid (on dry cardoon) in a 6L reactor provided with liquor recirculation, temperature control and sampling. Liquid samples were taken at different time and sugars analyzed by HPLC (Hi-Plex-H column). Solid fraction were removed at the end of the experiments, washed, weighed and enzymatic hydrolyzed with a mixture of commercial cellulases (Celluclast 1.5 L plus Novozym 188). The sugars in the liquefied fraction were analyzed by HPLC.

Under the conditions evaluated, the maximum glucose recovery was obtained when the cardoon was pretreated for 120 min. (maximum) at 190 °C (maximum) with a liquor-cardoon ratio of 30L/Kg (maximum) and with a 0.33% of ethanol (medium). These conditions also suppose the maximum delignification. However, taken into account the standard error, only temperature has a meaningful effect on the enzymatic saccharification and the conditions to obtain the best glucose yield at lower energy input are 60 min., 190°C, 20 L/Kg and 0.25% of ethanol. In these conditions, about a 90% of glucans are recovered in the pretreated solid, while a 70% of xylans are dissolved in the hydroalcoholic liquor. After enzymatic saccharification, a moderate glucose yield (60%) is obtained.

Paper-ID 75671

Paper title STRUCTURAL CHARACTERIZATION OF LIGNIN OBTAINED FROM TWO SOURCES AND ITS DEGRADATION BY BASIDIOMYCETE FUNGI

Presentation format Oral presentation

Date of submission 27.02.2017 05:30 Uhr

Authors - José Martín Carlos-Pérez (*josemartin.carlos@hotmail.com*) (Presenter)
- Jaime Saucedo-Luna (*saucedol@hotmail.com*)
- Agustín Jaime Castro-Montoya (*ajcastrofiq@gmail.com*)
- Lizbeth Pasaye-Anaya (*anayapl31@gmail.com*)
- Gerardo Vázquez-Marrufo (*gvazquezmarrufo@yahoo.com.mx*)

Content

Biofuels production from lignocellulosic biomass has been increasing within the last years, being recognized as the fourth source of energy and providing the 14% of the world energy consumption [1]. In Mexico, during 2016 there were produced more than 376 thousands of tons of agave bagasse, only from the tequila industry and INEGI has reported that the paper and pulp industry has produced more than 140 thousands of tons of lignin (LIG) that could be extracted from the black Kraft liquor [2, 3]. The aim of this work was to determine the structural characterization of Kraft lignin (LNK), agave bagasse lignin (LBA) and kraft alkali technical lignin (LTC). It also was studied the action of *Trametes versicolor*, *Phlebiopsis sp.* and *Irpex lacteus* over LBA and LTC. LNK was obtained by acid precipitation of Kraft black liquor and LBA was extracted from acid hydrolysis of agave bagasse. Elemental, IR and RMN analysis were carried out. Radial growth rate (RGR) and products generation determinations were performed in dextrose0.5%-lignin1% broth. Lignins showed similar bands within the IR spectrum (fig. 1) and some differences in NMR analysis (fig. 2) such as the S/G ratio. Elemental analysis, C9 formula, molecular weight, carboxyl and hydroxyl contents are described (table 1). Biodegradation assays demonstrate that LIG affects RGR (table 2) and their lignolytic activity is evident due to an oxidation halo (fig. 3). Further results will be obtained due to the dry weight, enzyme kinetics and HPLC analysis. As a manner of preliminary conclusions, it is state that LIG composition depends evidently on its source and extraction method, those analysis could help to determine further modifications in order to make it suitable as a building block for another processes. By the moment, the degradation on solid media showed ligninolytic activity that could lead, on submerged media analysis, to the production of value-added products.

References:

1. Álvarez-Castillo, A. et. al. Aprovechamiento integral de los residuos agroindustriales. Revista Iberoamericana de Polímeros, 2012. 13 (4): p. 140-150.
2. Consejo Regulador del Tequila. Date: February 23rd, 2017 (<https://www.crt.org.mx/>).
3. Instituto Nacional de Estadística y Geografía. Date: February 23rd, 2017 (www.inegi.org.mx/)

Table 1 Compositional characterization of lignin

	Elemental analysis				Functional groups content			S/G ratio	MW _{app}	
	%C	%H	%N	%S	%O	%OCH ₃	%OH-Ar			%OH-R
LTC	45.39	5.15	0.08	3.87	44.45	9.623	ND	ND	2.003.67	223.941
LKR	49.62	5.61	0.12	3.88	37.48	9.773	2.375	4.900	2.903.82	197.883
LBA	36.31	5.73	0.59	3.64	48.41	8.680	1.189	3.733	1.322.24	271.057

C9 expanded formula

LTC	$C_{12}H_{10.00}O_{3.11}(OCH_3)_{0.00}$
LKR	$C_{12}H_{10.02}O_{3.11}(OCH_3)_{0.16}$
LBA	$C_{12}H_{11.87}O_{3.03}(OCH_3)_{0.05}$

ND = Not determined, MW_{app} = Molecular weight of the phenylpropane unit (g/mol)

Table 2 Radial growth rate (RGR) of three basidiomycete

Media / Fungi	<i>Trametes versicolor</i>	<i>Phlebiopsis sp.</i>	<i>Irpex lacteus</i>
PDA	0.130	0.132	0.238
AEM	0.184	0.130	0.293
ADL	0.064	0.063	0.070
PDL	0.091	0.088	0.110
CZL	0.081	0.086	0.072
ADL2	0.145	0.110	0.135

