ΕΘΝΙΚΟ ΜΕΤΣΟΒΙΟ ΠΟΛΥΤΕΧΝΕΙΟ

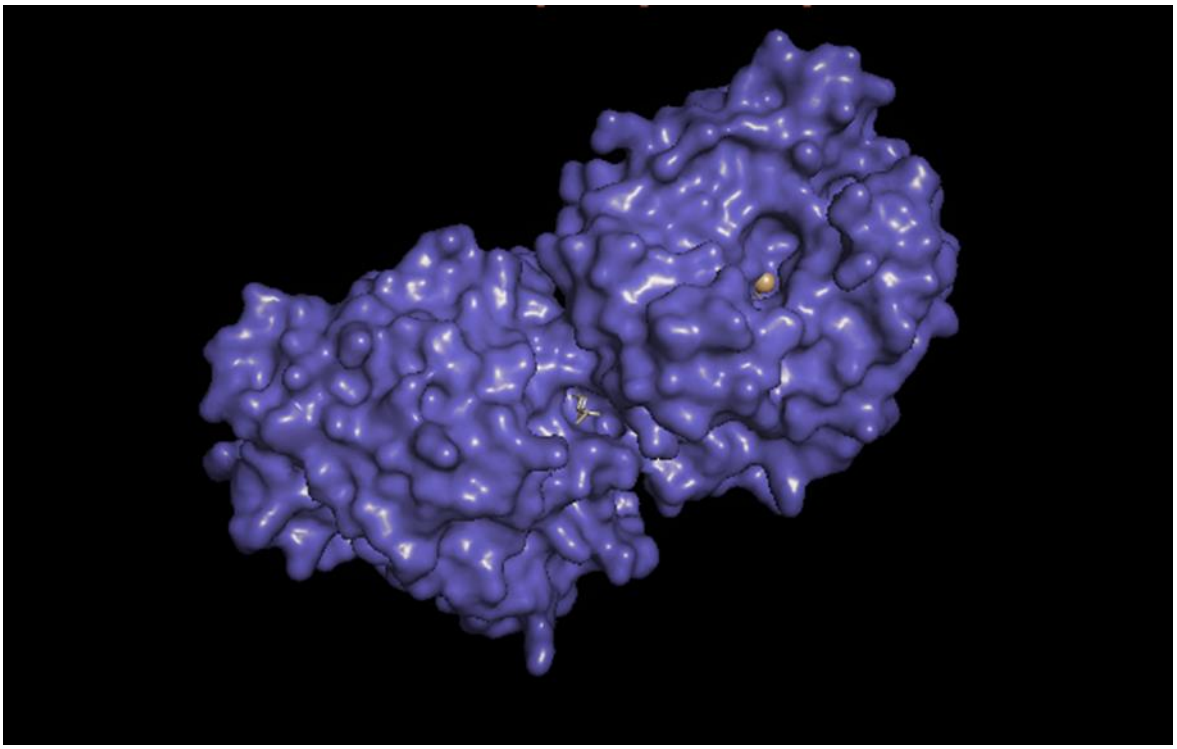
ΣΧΟΛΗ ΧΗΜΙΚΩΝ ΜΗΧΑΝΙΚΩΝ

ΤΟΜΕΑΣ IV: ΣΥΝΘΕΣΗ ΚΑΙ ΑΝΑΠΤΥΞΗ ΒΙΟΜΗΧΑΝΙΚΩΝ ΔΙΑΔΙΚΑΣΙΩΝ

ΕΡΓΑΣΤΗΡΙΟ ΒΙΟΤΕΧΝΟΛΟΓΙΑΣ

ΔΙΠΛΩΜΑΤΙΚΗ ΕΡΓΑΣΙΑ

ENZYME ASSISTED ABSORPTION OF CO_2 IN AQUEOUS SOLUTIONS OF ALKANOLAMINES AND AMINO ACID IONIC LIQUIDS



ΒΛΑΣΟΠΟΥΛΟΣ ΔΗΜΗΤΡΙΟΣ

Επιβλέπων: Ε. Τόπακας, Επίκουρος Καθηγητής ΕΜΠ

ΑΘΗΝΑ 2019

To my family and friends

ACKNOWLEDGEMENTS

This thesis project took place, in the framework of Erasmus program, in the laboratory of the department of Civil, Environmental and Natural Resources Engineering of the Luleå University of Technology (LTU), Sweden.

I would like to express my warm thanks to my supervisor from LTU, Professor Paul Christakopoulos, and my supervisor from National Technical University of Athens (NTUA), Assistant Professor Evangelos Topakas for the opportunity they have given me to carry out a thesis with such an interesting and important subject.

I would also like to thank my supervisor in the laboratory, senior Lecturer Magnus Sjöblom, for supporting me and guide me through the experimental process. I thank him for all the knowledge he offered me and the constant interest in my progress.

I want to thank my family and close friends for the continuous psychological and financial support they offered me during my stay in Sweden but also during these five years of my studies in the NTUA.

Last but not least, I want to thank everybody in the lab group for the cooperation they have offered me in an international and friendly environment.

TABLE OF CONTENTS

ANEX IN GREEK

ΠΕΡΙΛΗΨΗ

Το διοξείδιο του άνθρακα (CO_2) είναι η κύρια αιτία της υπερθέρμανσης του πλανήτη που καλύπτει περισσότερο από το 60% των αερίων θερμοκηπίου. Η χημική απορρόφηση είναι μια αποτελεσματική και ευρέως χρησιμοποιούμενη από τις βιομηχανίες μέθοδος για τον διαχωρισμό και τη δέσμευση του διοξειδίου του άνθρακα, χάρης στο χαμηλό κόστος στον εξοπλισμό, της υψηλής απόδοσης, των σταθερών συνθηκών λειτουργίας και της ώριμης τεχνολογίας. Ως κύριος διαλύτης στη βιομηχανία χρησιμοποιούνται οι αλκανολαμίνες οι οποίες προσφέρουν υψηλές ταχύτητες απορρόφησης. Ανάλογα με το είδος της αμίνης (πρωτογενής, δευτεροταγής, τριτοταγής), παρατηρούνται υψηλότεροι ρυθμοί απορρόφησης σε σειρά για τα υδατικά διαλύματα, 25% κατά βάρος, της μονοαιθανολαμίνη (MEA), ακολουθούμενη από την πενταιθυλενοεξαμίνη (PEHA) και τελευταίας της μεθυλοδιαιθανολαμίνη (MDEA). Ως διαλύτες ή σε μίγμα με αμινοδιαλύματα μπορούν να χρησιμοποιηθούν, επίσης, τα ιοντικά υγρά που προσφέρουν σταθερότητα και χαμηλότερη μεταβλητότητα στο σύστημα. Η προσθήκη βιοκαταλυτών στο σύστημα, όπως είναι το ένζυμο της ανθρακικής ανυδράσης, αυξάνουν τον ρυθμό απορρόφησης. Η ανθρακική ανυδράση (CA) είναι ένα ισχυρό μεταλλοένζυμο ψευδαργύρου που επιταχύνει τη μετατροπή του διοξειδίου του άνθρακα σε όξινο ανθρακικό ιόν. Μία γενετικά τροποποιημένη εκδοχή της ανθρακικής ανυδράσης έχει ενεργότητα της τάξης των 4,5 unit/μL ενζυμικού διαλύματος ή 2,07 units/mg λυοφιλιομένου ενζύμου, την οποία διατηρεί σε ικανοποιητικό βαθμό όταν βρεθεί σε υψηλές θερμοκρασίες και βασικό pH.

Σε μια διαδικασία απορρόφησης σε μικρής κλίμακας εξοπλισμό η μέγιστη χωρητικότητα σε διοξείδιο του άνθρακα διαφέρει μεταξύ των αμινών με την χωρητικότητα για το MDEA να φτάνει τα 80.7 $mgCO_2$/g διαλύτη, για το PEHA τα 122 $mgCO_2$/g διαλύτη, για το MEA τα 109,5 $mgCO_2$/g διαλύτη και για το ιοντικό υγρό PEHA-προλίνη να φτάνει τα 82.7 μgCO_2/g διαλύτη. Οι ρυθμοί απορρόφησης για τα υδατικά διαλύματα, 25% κατά βάρος, των MDEA, PEHA, MEA και PEHA-προλίνη υπολογίστηκαν σε 0.75 $mgCO_2$/(g διαλύτη*min), 2.79 $mgCO_2$ /(g διαλύτη*min), 3.12 $mgCO_2$/(g διαλύτη*min) και 1.64 $mgCO_2$/(g διαλύτη*min) αντίστοιχα. Με την προσθήκη ενζυμικού διαλύματος, 2% κατά βάρος στα παραπάνω

υδατικά διαλύματα, παρατηρήθηκε αύξηση στον ρυθμό απορρόφησης κατά 40% για το MDEA, 9.32% για το PEHA, 8.01% για το MEA και 4.88% για το PEHA-προλίνη.

Σε μεσαίας κλίμακας εξοπλισμό, υπολογίστηκαν οι ρυθμοί απορρόφησης και εκρόφησης αλκανολαμινών και των μειγμάτων μεταξύ τους και με ιοντικά υγρά, προσφέροντας σημαντικά αποτελέσματα. Συγκεκριμένα, το μείγμα MDEA-PEHA, σε αναλογία 1:1, παρουσίασε υψηλότερο ρυθμό σε σχέση με τον ρυθμό απορρόφησης του διαλύματος MDEA, πλησιάζοντας τον ρυθμό απορρόφησης του διαλύματος PEHA. Το μίγμα (PEHA-προλίνη)-MDEA σε αναλογία 1:1 παρουσίασε επίσης υψηλό ρυθμό απορρόφησης. Πειράματα πραγματοποιήθηκαν επίσης σε πιλοτική εγκατάσταση για υδατικό διάλυμα MDEA, 25% κατά βάρος, χωρίς ένζυμο.

I. Εισαγωγή

Το διοξείδιο του άνθρακα (CO_2) έχει αποδειχθεί ότι συμβάλλει σημαντικά στην υπερθέρμανση του πλανήτη, καλύπτοντας πάνω από το 60% των αερίων του θερμοκηπίου. Οι μισές εκπομπές CO_2 παράγονται από βιομηχανίες και σταθμούς παραγωγής ηλεκτρικής ενέργειας που χρησιμοποιούν ορυκτά καύσιμα. Ο ρόλος του CO_2 ως αερίου του θερμοκηπίου έχει οδηγήσει σε αυστηρότερους περιβαλλοντικούς κανονισμούς σχετικά με τις εκπομπές του στην ατμόσφαιρα. Έτσι, η επίτευξη λύσης για τη μείωση των εκπομπών CO_2 έχει μεγάλο ενδιαφέρον. Η δέσμευση μετά την καύση και η καύση με καθαρό οξυγόνο αντί για αέρα είναι οι κύριες τεχνολογίες μείωσης του εκπεμπόμενου CO_2.

Η χημική απορρόφηση είναι μία από τις πιο αποτελεσματικές μεθόδους για το διαχωρισμό και τη δέσμευση του CO_2. Το χαμηλό κόστος εξοπλισμού, η υψηλή απόδοση απομάκρυνσης, οι σταθερές συνθήκες λειτουργίας και το ώριμο τεχνολογικό υπόβαθρο θέτουν την χημική απορρόφηση ως την πιο δημοφιλή μέθοδο, η οποία εφαρμόζεται ευρέως στη χημική μηχανική, στη βιομηχανία τροφίμων και σε άλλους τομείς. Από τις υπάρχουσες τεχνολογίες που χρησιμοποιούνται, η χημική απορρόφηση είναι η πιο γνωστή τεχνική στη δέσμευση του CO_2 μετά την καύση, πιθανώς λόγω του μεγάλου αριθμού μονάδων παραγωγής ηλεκτρικής ενέργειας και των πολλά υποσχόμενων αποτελεσμάτων μέχρι στιγμής. Όσον αφορά την επιλογή των διαλυτών, οι αμίνες παραδοσιακά θεωρούνται ως βέλτιστα αντιδραστήρια επιλογής. Στην φύση η απορρόφηση του διοξειδίου του άνθρακα απαντάται πολύ συχνά στα υδάτινα οικοσυστήματα (λίμνες, ποτάμια κλπ.) δημιουργώντας ένα σύστημα ονομαζόμενο ανθρακικό σύστημα, το οποίο παίζει πρωτεύοντα ρόλο στην πρόσληψη άνθρακα από παραγωγικούς φωτοσυνθετικούς οργανισμούς και από άλλους αυτοτροφικούς οργανισμούς και ελέγχει την συσσώρευση του διοξειδίου του άνθρακα στην ατμόσφαιρα.

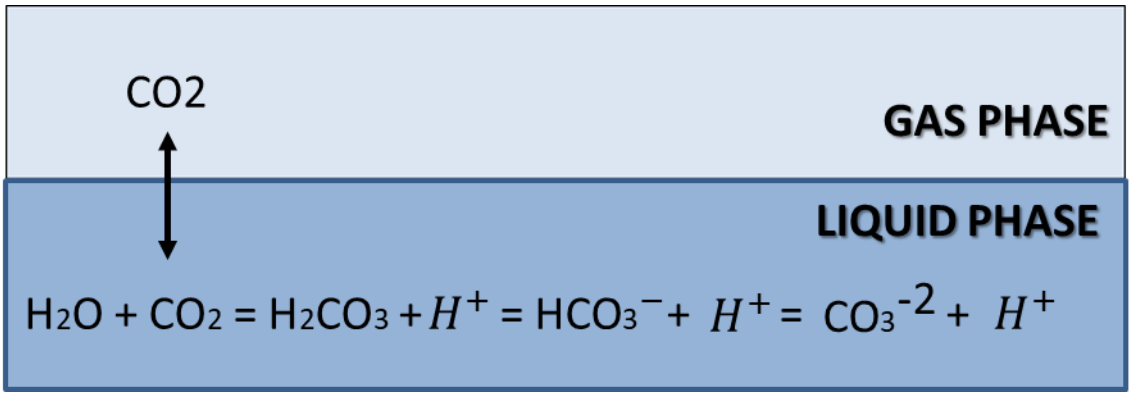


Figure I.1: Ανθρακικό σύστημα

Στην περίπτωση της χρήσης της χημικής απορρόφησης στην βιομηχανία η υγρή φάση δεν αποτελείται από νερό αλλά από υδατικά διαλύματα αμινών τα οποία αυξάνουν τον ρυθμό απορρόφηση αλλάζοντας το ανθρακικό σύστημα ανάλογα με το είδος των αμινών. Για ένα διάλυμα πρωτοταγούς ή δευτεροταγούς αμίνης το σύστημα αποτελείται από τις αντιδράσεις όπως αυτές παρουσιάζονται παρακάτω:

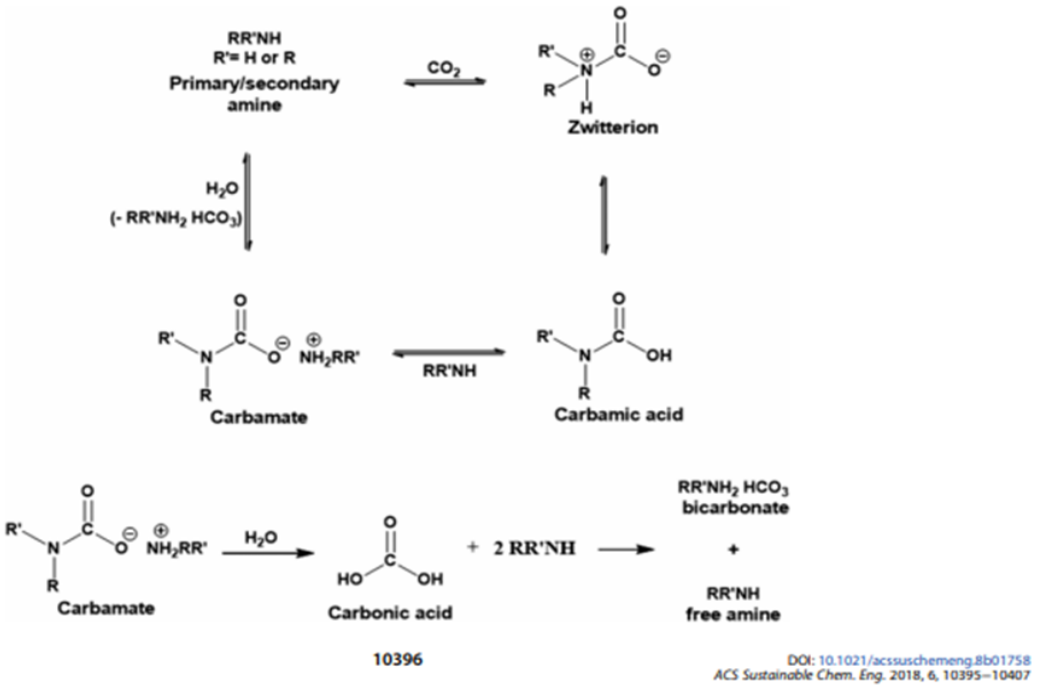


Figure I.2 Σύστημα αντιδράσεων πρωτοταγούς/δευτεροταγούς αμίνης με CO_2

Χαρακτηριστικό παράδειγμα είναι η μονοαιθανολαμίνη, η οποία χρησιμοποιείται ευρέως σε διεργασίες απορρόφησης CO_2. Στην περίπτωση μια τριτοταγούς αμίνης όπως είναι η N-Μεθυλδιαιθανολαμίνη, το σύστημα αδυνατεί να σχηματίσει καρβαμικό ιόν, με αποτέλεσμα την βραδύτερη αντίδραση με το CO_2, ακολουθώντας τις παρακάτω αντιδράσεις:

$$H_2O + R_3N \leftrightarrow OH^- + R_3NH^+ \text{ (στιγμιαία)}$$

$$OH^- + CO_2 \leftrightarrow HCO_3^- \text{ (αργή)}$$

Η συνολική αντίδραση περιλαμβάνει επίσης τον σχηματισμό ανθρακικού οξέος και διττανθρακικού:

$$\text{Σχηματισμός ανθρακικού οξέος: } CO_2 + H_2O \leftrightarrow H_2CO_3$$

$$\text{Σχηματισμός διττανθρακικού: } CO_2 + OH^- \leftrightarrow HCO_3^-$$

$$RNH_2^+COO^- + RNH_2 \rightleftharpoons RNHCOO^- + RNH_3^+$$

Τέλος, μία ακόμη πολλά υποσχόμενη αμίνη είναι η πενταιθυλενεξαμίνη η οποία έχει δύο πρωτοταγείς και τέσσερις δευτεροταγείς αμινομάδες στη δομή της. Λόγω αυτής της υψηλής περιεκτικότητας σε αμινομάδες ανά μονάδα μάζας, της υψηλής θερμικής σταθερότητας και της χαμηλής τοξικότητάς της, μπορεί να αποτελέσει καλό υποψήφιο για τη δέσμευση CO_2.

Θετικά σε αμινικά διαλύματα μπορούν να λειτουργήσουν τα ιοντικά υγρά. Πρόκειται για οργανικά άλατα που σε σύγκριση με τις αλκανολαμίνες, έχουν το πλεονέκτημα της χαμηλής πτητικότητας και της σημαντικής θερμικής σταθερότητας. Με την εισαγωγή τους στο διάλυμα θα μπορούσαν να ενισχύσουν την απορρόφηση του CO_2. Η αντίδραση ενός ιοντικού υγρού με το CO_2 παρουσιάζεται παρακάτω:

Figure I.3: Αντίδραση ιοντικών υγρών με CO_2

Από τεχνολογική άποψη, μια τυπική διαδικασία χημικής απορρόφησης αποτελείται από μια στήλη απορρόφησης και μία στήλη εκρόφησης, στην οποία το απορροφητικό υλικό αναγεννάται θερμικά. Σε μια διαδικασία χημικής απορρόφησης, το καυσαέριο που περιέχει CO_2 εισέρχεται σε μία κλίνη με πληρωτικό υλικό, από τον πυθμένα και έρχεται σε επαφή με υγρό απορρόφησης αντίθετης ροής. Μετά την απορρόφηση, το πλούσιο σε CO_2 υγρό ρέει σε κλίνη εκρόφησης για θερμική αναγέννηση. Μετά την αναγέννηση, το υγρό απαλλαγμένο από το CO_2 διοχετεύεται ξανά στην κλίνη απορρόφησης δημιουργώντας έναν κυκλικό τρόπο λειτουργίας. Το καθαρό CO_2 που απελευθερώνεται από την κλίνη εκρόφησης στη συνέχεια συμπιέζεται για μεταφορά και αποθήκευση.

Ένας ακόμη τρόπος βελτίωσης του ρυθμού απορρόφησης είναι η χρήση βιοκαταλυτών (ένζυμα) στο αμινοδιάλυμα. Ένα τέτοιο ένζυμο είναι η ανθρακική ανυδράση (CA), ένα ισχυρό μεταλλοένζυμο ψευδαργύρου που επιταχύνει τη μετατροπή του διοξειδίου του άνθρακα σε διττανθρακικό. Η γενετική τροποποίηση αυτού του ενζύμου καθιστά δυνατή τη χρήση του σε συνδυασμό με υδατικά διαλύματα αλκανολαμίνης σε ένα βιομηχανικό περιβάλλον, όπως η επεξεργασία καυσαερίων. Ο μηχανισμός λειτουργίας του ενζύμου παρουσιάζεται παρακάτω:

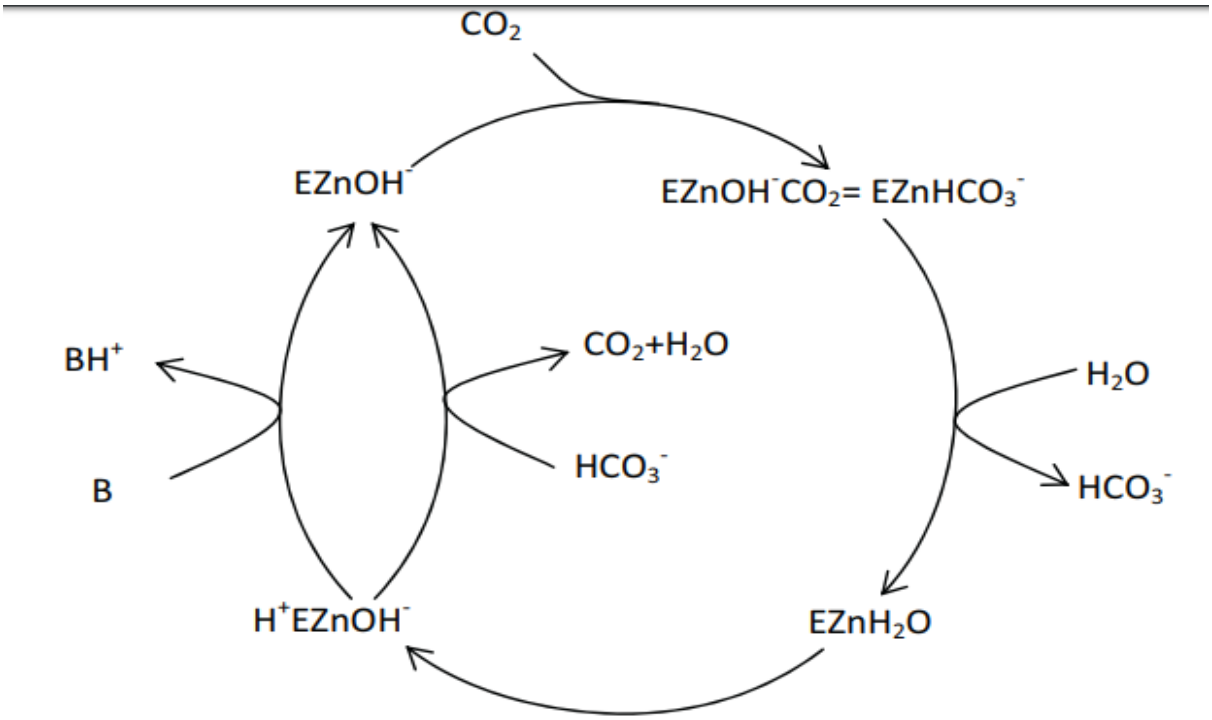


Figure I.4: Μηχανισμός λειτουργίας της ανθρακικής ανυδράσης

II. Υλικά και μέθοδοι

Υλικά

Αλκανολαμίνες

Οι αλκανολαμίνες και το αμινοξύ προλίνη προμηθεύτηκαν από την Sigma-Aldrich. Τα αμινικά διαλύματα που χρησιμοποιήθηκαν παρασκευάσθηκαν με αραίωση κατά βάρος.

Μικροοργανισμοί και μέσα ανάπτυξης

Για την έκφραση της ανθρακικής ανυδράσης χρησιμοποιήθηκαν ανασυνδυασμένα κύτταρα E. coli. Η ανασυνδυασμένη γονιδιακή αλληλουχία σχηματίστηκε σύμφωνα με βιβλιογραφία και δόθηκε σε ιδιωτική εταιρεία για τον ανασυνδυασμό των κυττάρων. Κατά την έκφραση του γονιδίου χρησιμοποιήθηκαν δύο μέσα ανάπτυξης. Το πρώτο είναι το Luria Broth από την Sigma Company (L3522) και το δεύτερο το ZYP-5052. Για την παρασκευή του μέσου ZYP-5052 χρησιμοποιήθηκαν τέσσερα stock διαλύματα.

<u>Stock Διαλύματα</u>

ZY		20xNPS		50x5052	
H_2O		$(NH_4)_2SO_4$	66g/L	γλυκερόλη	250 g/L
Yeast extract	5g/L	KH_2PO_4	136 g/L	γλυκόζη	25 g/L
Tryptone	10g/L	Na_2HPO_4	142 g/L	λακτόζη	100 g/L

1000xTrace elements: 100mL stock solution				
Salt	Concentration	g/mg/ml	MW	Special preparation
$FeCl_3$ x $6H_2O$	50mM	50 ml of 0.1 M stock	270	0.1M stock (V = 100ml) 2.7 g in 1:100 diluted HCl
$CaCl_2$ x2 H_2O	20 mM	0.294 g	147	
$MnCl_2$ x4 H_2O	10 mM	0.198 g	198	
$ZnSO_4$ x$7H_2O$	10 mM	0.288 g	288	
$CoCl_2$ x$6H_2O$	2 mM	47.6 mg	238	
$CuCl_2$ x$2H_2O$	2 mM	34 mg	171	
$NiCl_2$ x$6H_2O$	2 mM	47.5 mg	238	
Na_2MoO_4 x$2H_2O$	2 mM	48.4 mg	242	
H_3BO_3	2mM	400µL of 0.5M stock	61.5	0.5M stock (V= 50ml) = 1.53 g in ca 60mM HCL

Για την παρασκευή 1 lt of ZYP-5052 απαιτούνται:

- 928ml ZY
- 1ml MgSO4 (1M)
- 1ml 1000xTrace elements
- 20ml 50x5052
- 50ml 20xNPS

Συσκευές και όργανα

Οι συσκευές και τα όργανα που χρησιμοποιήθηκαν είναι:

Microplate spectrometer reader	Vacuum filtration apparatus
Spectromax M2 Spectrophotometer	Centrifuge
pH-meter	Rotameter
Incubator	Flowmeter
Magnetic Stirrer	Thermometer
Hotplate	Manometer
Millie Q water device	CO_2, N_2 tank
Pump	Autoclave

Εκτός από τα παραπάνω όργανα, χρησιμοποιήθηκαν διάφορα εργαστηριακά υλικά, όπως ογκομετρικοί κύλινδροι, ποτήρια, κωνικές φιάλες, χοάνες, πιάτα, δοχεία Eppendorf, πιπέτες και γυάλινες φιάλες.

Μέθοδοι

Παραγωγή ανθρακικής ανυδράσης και μέθοδοι αντοχής της σε υψηλές θερμοκρασίες και ακραίες τιμές pH

Το E. coli αναπτύχθηκε σε μέσο L. Broth (25 g/L) με χρήση του αντιβιοτικού αμπικιλλίνη (100 μg / ml) στους 32° C για 6 - 8 ώρες. Έγινε ανακαλλιέργεια σε 0,5L ZYP-5052 στους 32° C για 10 ώρες. Ακολούθησε φυγοκέντρηση σε 10000 rpm για 10min. Το ίζημα (κύτταρα) αραιώθηκε με διάλυμα bug buster κατ' αναλογία προς το υπερκείμενο 17:1 και προστέθηκαν 1-2 μL lysonase. Για το λυοφιλιομένο δείγμα ενζύμου, το ίζημα διαλύθηκε σε ρυθμιστικό διάλυμα Tris-HCl (0.1M και pH8.3) και 0.1M NaCl και ακολούθησε ομογενοποίηση στα 700bar για 3 κύκλους. Το ομογενοποιημένο διάλυμα στη συνέχεια λυοφιλιώνεται.

Η ενεργότητα του ενζύμου υπολογίσθηκε με τιτλομετρική μέθοδο κατά την οποία υπό ψυχρές συνθήκες (0^oC) αναμιγνύονται 5μL υγρού ενζύμου ή 10 mg ξηρού με 1 ml ρυθμιστικού διαλύματος Tris-HCL 0,01 M (pH 8,3) και δείκτη BTB (BromoThymol Blue). Η μέτρηση ξεκινάει με προσθήκη 1 ml, κορεσμένου σε CO_2, νερού και σημειώνεται ο χρόνος που απαιτείται για την αλλαγή χρώματος ($T_{control}$) από μπλε σε κίτρινο. Ο μη ενζυματικός ρυθμός αντίδρασης μετρήθηκε αναμειγνύοντας το κορεσμένο σε CO_2 νερό με το ρυθμιστικό διάλυμα χωρίς το ένζυμο (T_{blank}). Η δοκιμασία επαναλήφθηκε 3 φορές. Το unit ανθρακικής ανυδράσης υπολογίστηκε ως (T_{blank} / $T_{control}$ - 1) και η ενεργότητα εκφράστηκε ως unit/μl ενζύμου ή unit/mg ενζύμου, όπου T_{blank} = χρόνος που απαιτείται για αλλαγή χρώματος του τυφλού, $T_{control}$ = χρόνος που απαιτείται για την αλλαγή του χρώματος του δείγματος.

Για να μετρηθεί η σταθερότητα της ανθρακικής ανυδράσης ως προς την θερμοκρασία και ως προς βασικό περιβάλλον υπολογίζεται η ενεργότητα του ενζύμου μέσω του ρυθμού της αντίδρασης της θερμικής αποσύνθεσης ανθρακικού καλίου στην οποία η ανθρακική ανυδράση χρησιμοποιείται ως καταλύτης.

$$KHCO_3 \rightarrow K_2CO_3 + CO_2 \uparrow + H_2O$$

Για να μετρηθεί ο ρυθμός της αντίδρασης χρησιμοποιείται φασματόμετρο με ορισμένο μήκος κύματος στα 550nm (A550), ρυθμιστικό διάλυμα 300 mM KHC03 (pH 8,0) και 1% φαινολοφθαλεΐνη ως δείκτης. Ένα διάλυμα μη ανασυνδυασμένων κυττάρων E. coli

χρησιμοποιήθηκε, ως λευκό διάλυμα, για τη μέτρηση της ταχύτητας της αντίδρασης χωρίς το ένζυμο. Η ενεργότητα υπολογίστηκε ως η διαφορά της ταχύτητας της αντίδρασης με ένζυμο και της μη καταλυτικής αντίδρασης. Η μέθοδος χρησιμοποιήθηκε και για τον έλεγχο της σταθερότητας του ενζύμου συνδυαστικά σε βασικό περιβάλλον και υψηλές θερμοκρασίες.

Απορρόφηση CO_2 σε αμινοδιαλύματα

Απορρόφηση σε εξοπλισμό μικρού κελύφους

Η μέθοδος βασίζεται στην πτώση πίεσης CO_2 από ένα σημείο εκκίνησης 300 mbar σε κλειστό θάλαμο στον οποίο περιέχεται διάλυμα αμίνης υπό ανάδευση (300rpm). Κατά τη διάρκεια του πειράματος ο θάλαμος πληρώνεται με 99,9% αέριο CO_2 και το διάλυμα αμίνης απορροφά το CO_2, προκαλώντας πτώση πίεσης στον θάλαμο. Η πτώση πίεσης καταγράφεται από ένα ηλεκτρονικό μανόμετρο συνδεδεμένο σε υπολογιστή. Τα δείγματα που εξετάστηκαν ήταν υδατικά διαλύματα αμίνης (MDEA, PEHA, MEA), 25% κατά βάρος με και χωρίς την παρουσία διαλύματος ενζύμου 2% κατά βάρος. Ο θάλαμος ζυγίζεται πριν και μετά την απορρόφηση. Ένα διάλυμα μη ανασυνδυασμένων κυττάρων E. coli χρησιμοποιήθηκε ως λευκό.

Απορρόφηση σε εξοπλισμό μεσαίας κλίμακας

Ο εξοπλισμός αποτελείται από τον κύριο θάλαμο, έναν συμπυκνωτή, έναν μετρητή ροής στην έξοδο και έναν αναλυτή CO_2. Για την ανάδευση χρησιμοποιήθηκε μαγνητικός αναδευτήρας. Η θερμοκρασία ελέγχθηκε με θερμόμετρο και διατηρήθηκε με θερμαντική επιφάνεια. Πριν την έναρξη, πραγματοποιείται βαθμονόμηση του αναλυτή με μείγμα αερίου CO_2-N_2 γνωστής συγκέντρωσης. Η θερμοκρασία απορρόφησης ήταν στους 40 ± 0,5 ^oC και η ανάδευση ρυθμίστηκε στα 300 rpm. Η παροχή εισόδου ήταν ρυθμισμένη στα 500 mL min^{-1}. Το διάλυμα αποτελείται από 100 ml διαλύτη αμίνης με και χωρίς ένζυμο. Η ποσότητα υγρού ενζύμου που προστέθηκε ήταν 1% κατά βάρος.

Το λυοφιλιομένο ένζυμο χρησιμοποιήθηκε επίσης σε διαφορετικές συγκεντρώσεις. Για την εκρόφηση χρησιμοποιείται ως φέρον αέριο N_2 (200 ml min^{-1}) και υπολογίζεται το ποσοστό

CO_2 στην έξοδο. Η θερμοκρασία εκρόφησης είναι 80 ± 0,5 °C. Ο ρυθμός απορρόφησης/εκρόφησης CO_2 υπολογίστηκε από την εξίσωση:

$$Q_{CO_2}\left[\frac{mol\ CO_2\ in\ solution}{s}\right] = \left(n_{CO_2}^{in}\left[\frac{mol\ CO_2}{s}\right] - \frac{x_{CO_2}^{out}[mole\ fraction]n_{N_2}\left[\frac{mol\ N_2}{s}\right]}{(1-x_{CO_2}^{out})[mole\ fraction]}\right)$$

Απορρόφηση σε πιλοτική κλίνη

Το διάλυμα αμίνης εισέρχεται από την κορυφή της στήλης. Για την απορρόφηση χρησιμοποιείται ένα μείγμα αέρα με 14,3% CO_2 από το κάτω μέρος της στήλης. Για την εκρόφηση χρησιμοποιήθηκε άζωτο (N_2) ως φέρον αέριο. Η ροή του διαλύτη ρυθμίστηκε στα 24.0 L/min, η ροή μίγματος αερίου ρυθμίστηκε στα 4.2 L / min και η ροή N_2 ρυθμίστηκε στα 2.3 L/min. Στην έξοδο το ποσοστό του CO_2 μετρήθηκε με αναλυτή CO_2. Η θέρμανση πραγματοποιήθηκε με λέβητα. Η θερμοκρασία απορρόφησης ρυθμίστηκε στους 40 °C και για την εκρόφηση στους 80 °C. Δείγματα ελήφθησαν από το μέσο της στήλης και αναλύθηκαν με τη μέθοδο της τιτλοδότησης (μέθοδος $BaCl_2$) για την μέτρηση του CO_2.

Η μέθοδος τιτλοδότησης (μέθοδος $BaCl_2$)

Το δείγμα αναμιγνύεται σε διάλυμα υδροξειδίου του νατρίου (NaOH, 0,1 mol L^{-1}) και χλωριούχου βαρίου ($BaCl_2$, 0,5 mol L^{-1}) σε αναλογία 2-1. Ακολουθεί θέρμανση για να ενισχυθεί ο σχηματισμός ανθρακικού βαρίου ($BaCO_3$), στη συνέχεια ψύξη σε θερμοκρασία περιβάλλοντος και διήθηση με χαρτί Millipore των 0,45 μm. Ακολουθεί έκπλυση του διηθητικού χαρτιού με απιονισμένο νερό και μεταφορά σε ποτήρι ζέσεως. Το ίζημα διαλυτοποιείται σε διάλυμα υδροχλωρίου (HCl, 0.1 mol L^{-1}) και το μείγμα τιτλοδοτείται με NaOH, 0,1 mol L^{-1}, με τελικό σημείο pH 5.2 (δείκτης).

III. Αποτελέσματα και συζήτηση

Ενεργότητα ανθρακικής ανυδράσης

Η ενεργότητα του ενζύμου υπολογίσθηκε με τον τύπο Units = ($T_{blank}/T_{control}$ – 1) και τα αποτελέσματα παρουσιάζονται παρακάτω:

Table III-1: Ενεργότητα διαλύματος ενζύμου και λυοφιλιομένου ενζύμου ανθρακικής ανυδράσης

Ενεργότητα Ανθρακικής Ανυδράσης	Units	
Διάλυμα ενζύμου	22.57	4.5 units/μL
Λυοφιλιομένο ένζυμο	12.44	2.07 units/mg

Αντοχή της ανθρακικής ανυδράσης σε ακραίες συνθήκες θερμοκρασίας και pH

Η ενεργότητα του ενζύμου παραμένει σταθερή σε υψηλές θερμοκρασίες με όριο τους 90°C για τους οποίους το ένζυμο παρουσιάζει αδρανοποίηση μετά από χρόνο 1400min.

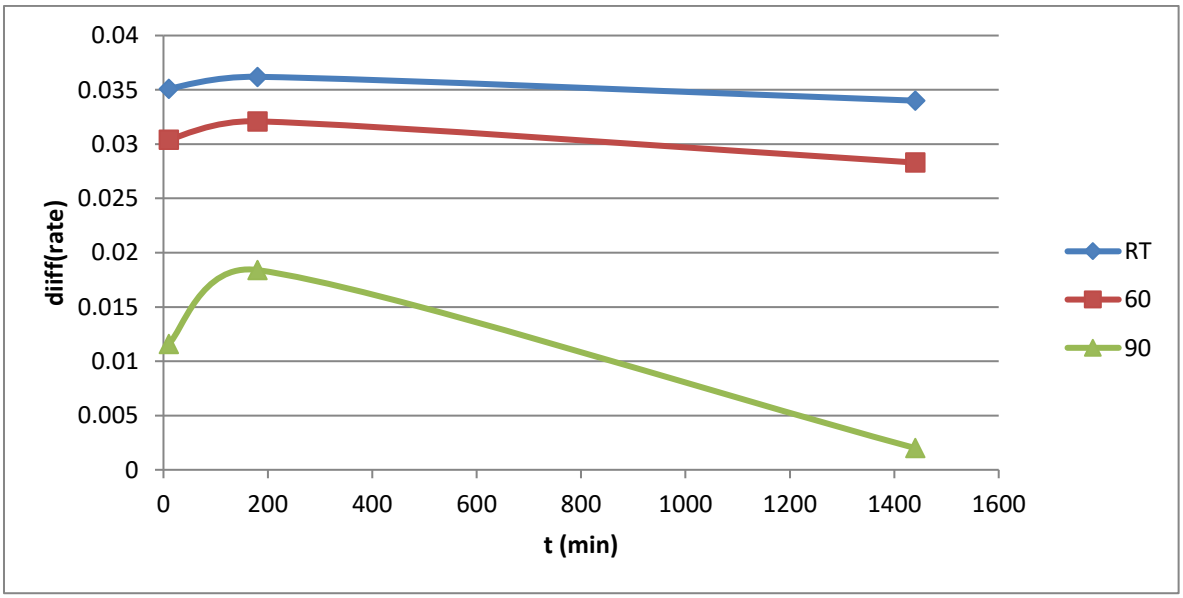


Figure III.5: Μεταβολή της ενεργότητας του ενζύμου σε βάθος χρόνου και σε θερμοκρασία δωματίου, 60°C και 90°C

Σε βασικό περιβάλλον το ένζυμο είναι σταθερό παρουσιάζοντας μια σταθερή ενεργότητα, χαμηλότερη ωστόσο από την ενεργότητα του σε ουδέτερο pH.

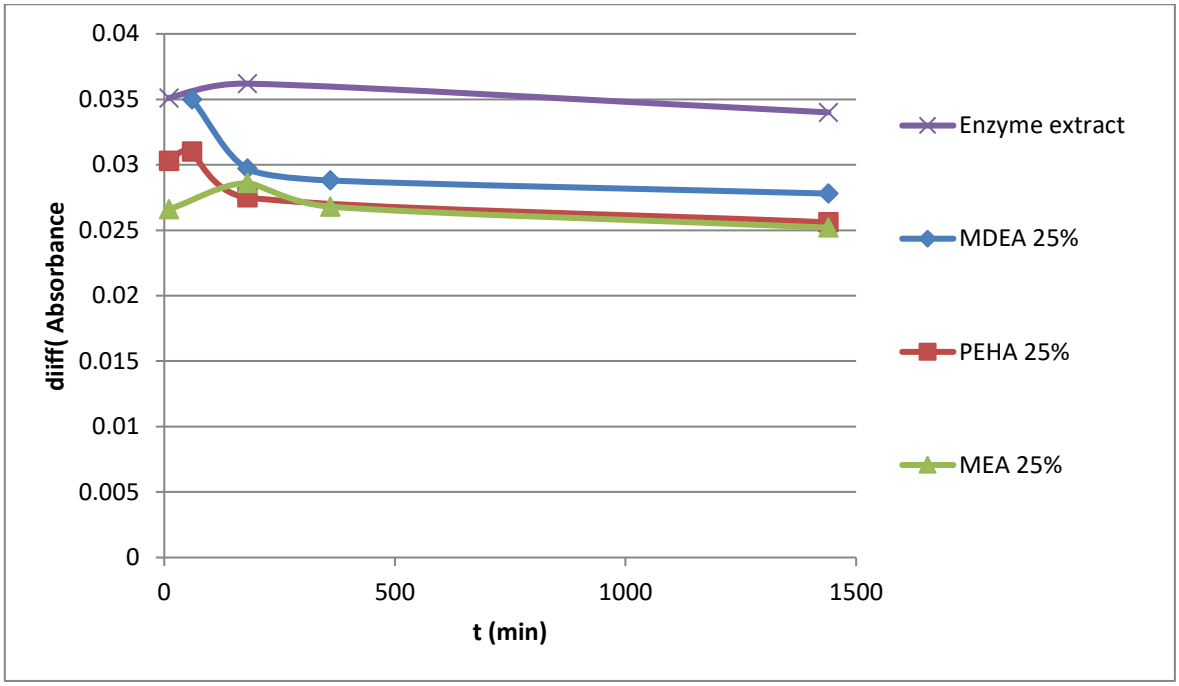


Figure III.6: Μεταβολή ενεργότητας ενζύμου σε ουδέτερο περιβάλλον και σε βασικό περιβάλλον προκαλούμενο από διάφορα αμινοδιαλύματα

Σε συνδυασμένες συνθήκες υψηλής θερμοκρασίας και βασικού pH, παρατηρείται μια μείωση της ενεργότητας με σταθερό ρυθμό με όριο τους 90°C στους οποίους σε όλα τα αμινοδιαλύματα το ένζυμο αδρανοποιείται.

Απορρόφηση CO_2 σε μικρής κλίμακας εξοπλισμό

Αρχικά πραγματοποιήθηκε έλεγχος της χωρητικότητας των αμινοδιαλυμάτων σε CO_2 η οποία παρέμεινε ίδια με και χωρίς την παρουσία ενζύμου.

Table III-2: Μέγιστη χωρητικότητα σε CO_2 υδατικών αμινοδιαλυμάτων 25% κατά βάρος

MDEA	PEHA	MEA	PEHA-προλίνη
80.7 mgCO_2/g sample	122 mgCO_2/g sample	109.5 mgCO_2/g sample	82.7 mgCO_2/g sample

Σε πειράματα για τον ρυθμό απορρόφησης προέκυψαν τα εξής αποτελέσματα:

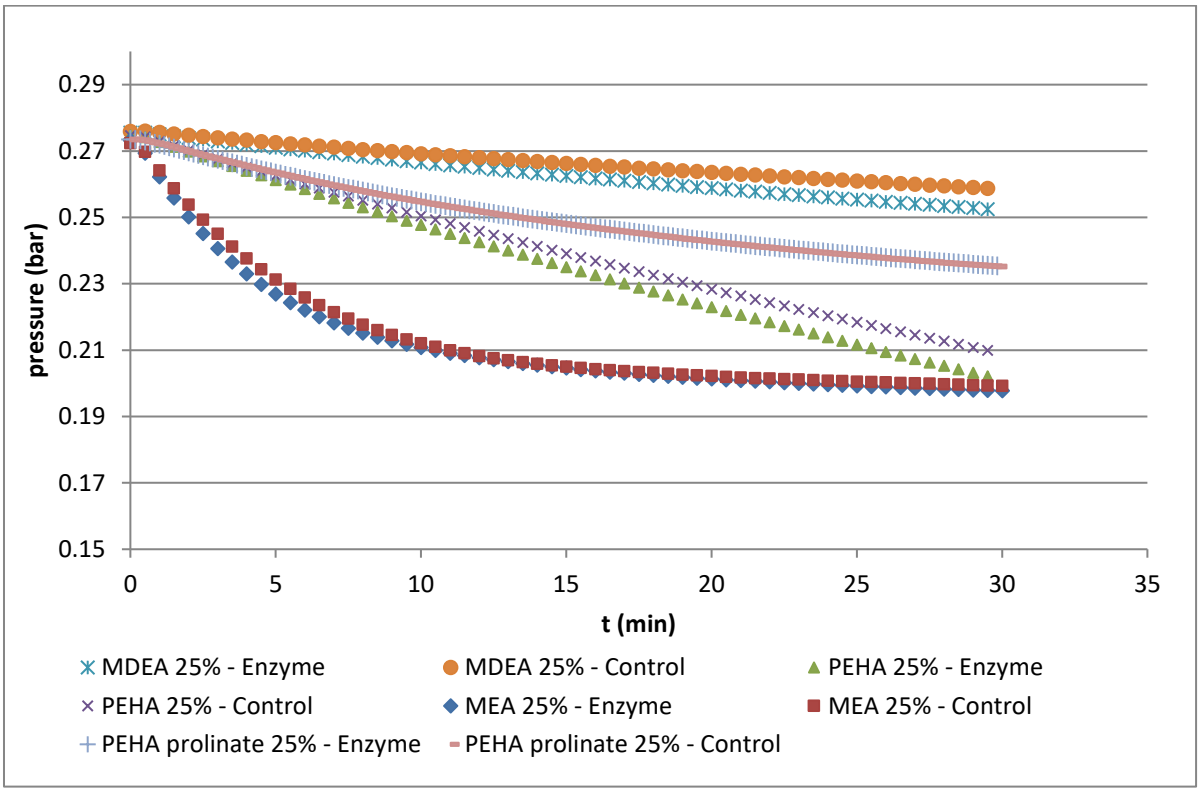


Figure III.7: Πτώση πίεσης σε κλειστό χώρο καθώς το αέριο CO_2 απορροφάται από δείγματα αμινοδιαλυμάτων με και χωρίς την παρουσία ένζυμο

Τα διαγραμματικά και τα βαρυμετρικά αποτελέσματα επιβεβαιώνουν την επιτάχυνση της απορρόφησης με την παρουσία του ενζύμου στα αμινοδιαλύματα με μόνη εξαίρεση το μίγμα αμίνης ιοντικού υγρού για το οποίο επιβεβαιώνεται μόνο βαρυμετρικά.

Table III-3: Ρυθμός απορρόφησης σε αμινοδιαλύματα 25% κατά βάρος με και χωρίς ένζυμο

	MDEA	PEHA	MEA	PEHA-προλίνη
Με ένζυμο	1.05 $mgCO_2/g$ sample/min	3.05 $mgCO_2/g$ sample/min	3.37 $mgCO_2/g$ sample/min	1.72 $mgCO_2/g$ sample/min
Χωρίς ένζυμο	0.75 gCO_2/g sample/min	2.79 gCO_2/g sample/min	3.12 gCO_2/g sample/min	1.64 gCO_2/g sample/min

Απορρόφηση CO_2 σε μεσαίας κλίμακας εξοπλισμό

Τα αποτελέσματα σε αυτόν τον εξοπλισμό επιβεβαιώνουν τον υψηλό ρυθμό απορρόφησης του MEA συγκριτικά με τα υπόλοιπα δείγματα. Χρησιμοποιήθηκαν δείγματα MEA συγκέντρωσης 25% και 6,5% κατά βάρος. Θετικά αποτελέσματα προκύπτουν και για το ιοντικό υγρό PEHA-προλίνη, ο ρυθμός απορρόφησης του οποίου πλησιάζει τον ρυθμό του MEA 25%. Εξετάζεται επίσης μίγμα αμινοδιαλυμάτων PEHA – MDEA σε αναλογία 1:1, με ενθαρρυντικά αποτελέσματα. Ο χαμηλότερος ρυθμός απορρόφησης παρατηρείται για το MDEA, ωστόσο αυτός βελτιώνεται σημαντικά με την προσθήκη ενζύμου.

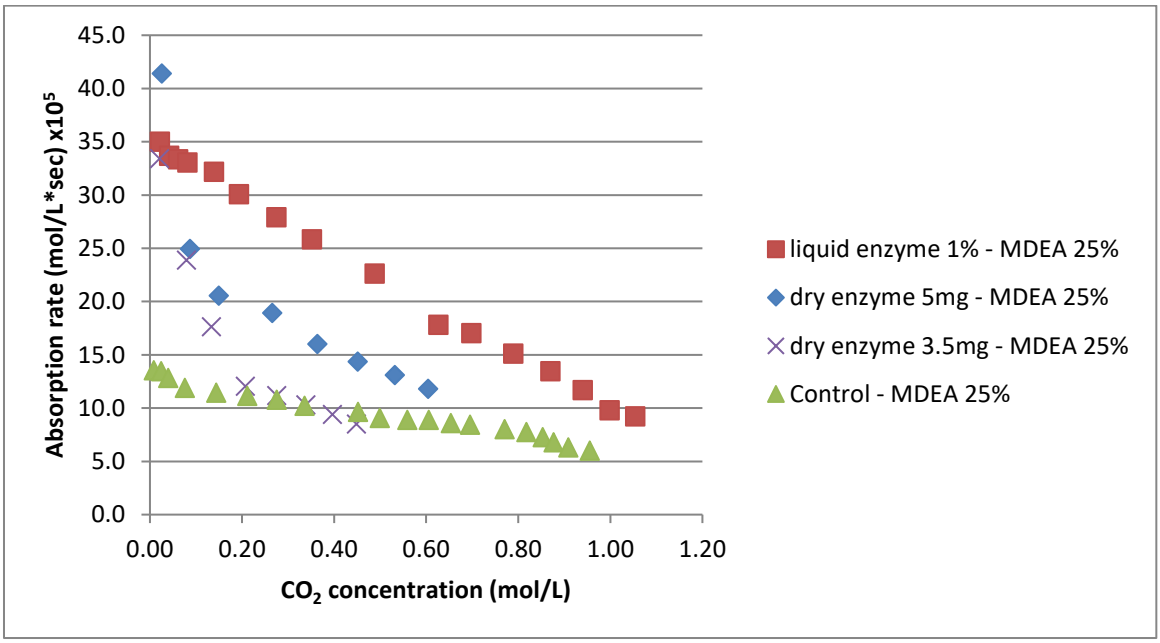


Figure III.8: Ρυθμός απορρόφησης CO_2 σε υδατικό διάλυμα MDEA 25% κατά βάρος με προσθήκη διαλύματος ενζύμου 1% κατά βάρος και λυοφιλιομένου ενζύμου

Το ένζυμο δρα θετικά για όλα τα αμινοδιαλύματα και τα ιοντικά υγρά με εξαίρεση το MEA συγκέντρωσης 25% κατά βάρος για το οποίο δεν παρατηρείται μεταβολή του ρυθμού απορρόφησης παρουσία ενζύμου, πιθανώς εξαιτίας της υψηλής συγκέντρωσης της αμίνης και του ήδη υψηλού ρυθμού που παρουσιάζει.

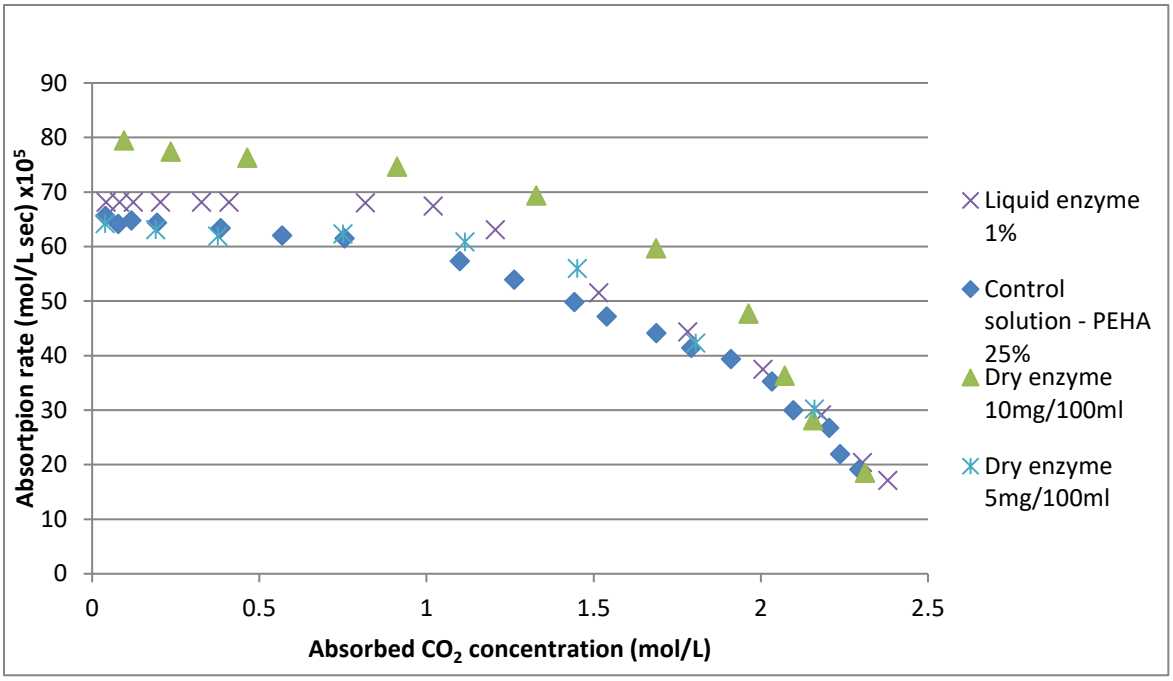


Figure III.9: Ρυθμός απορρόφησης CO_2 σε υδατικό διάλυμα PEHA 25% κατά βάρος με προσθήκη διαλύματος ενζύμου 1% κατά βάρος και λυοφιλιομένου ενζύμου

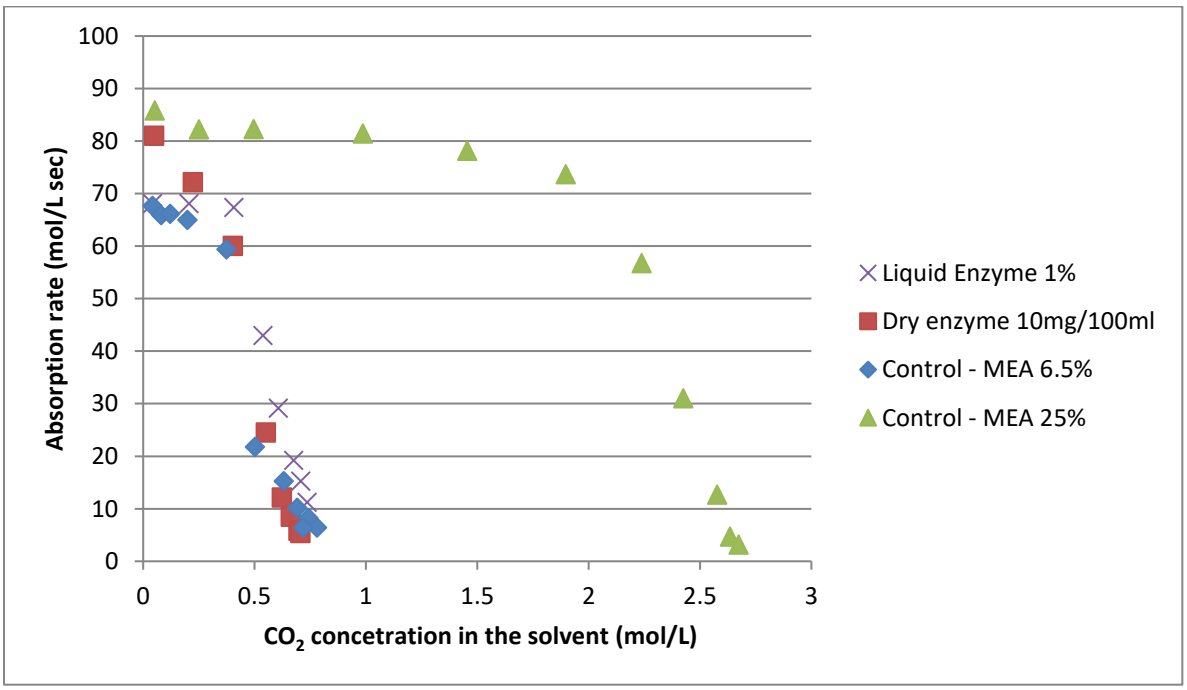


Figure III.10: Ρυθμός απορρόφησης CO_2 σε υδατικό διάλυμα MEA για συγκεντρώσεις 6,5% και 25% κατά βάρος με προσθήκη διαλύματος ενζύμου 1% κατά βάρος και λυοφιλιομένου ενζύμου

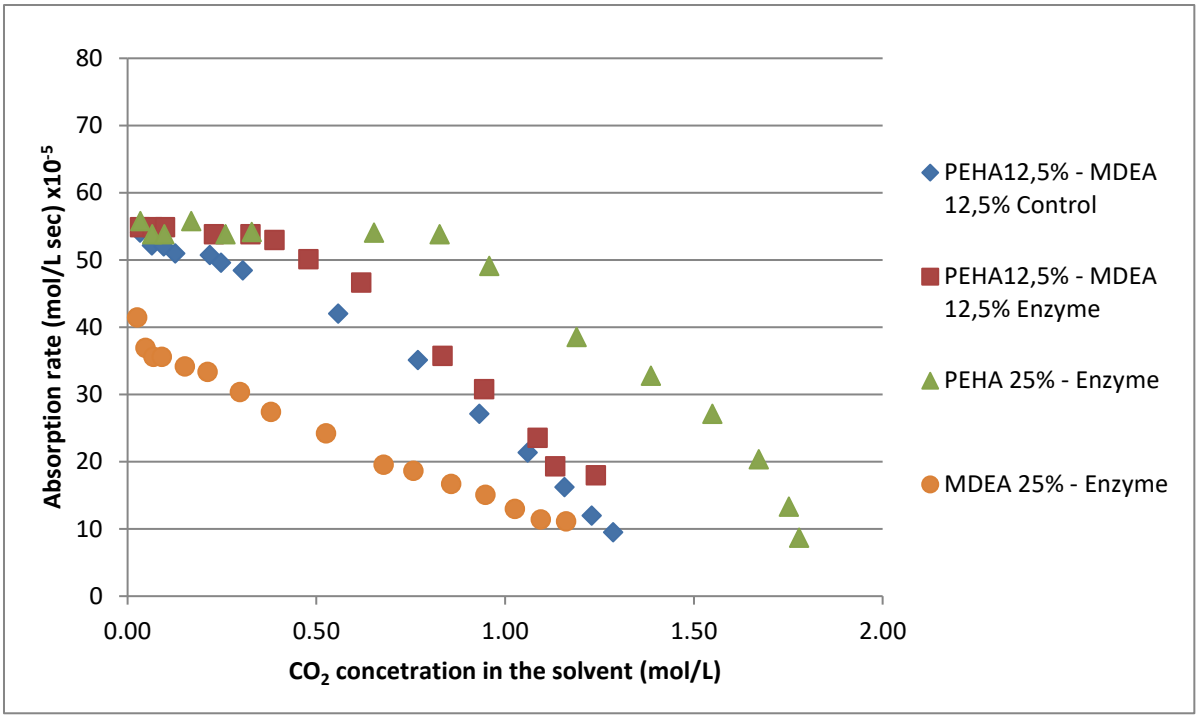


Figure III.11: : Ρυθμός απορρόφησης CO_2 σε μίγμα PEHA-MDEA 1:1 με προσθήκη διαλύματος ενζύμου 1% κατά βάρος και οι ρυθμοί απορρόφησης των PEHA και MDEA με προσθήκη διαλύματος ενζύμου 1% κατά βάρος

Στην διεργασία της εκρόφησης ο ρυθμός διατηρείται παρουσία ενζύμου. Εξαίρεση αποτελεί το διάλυμα με το PEHA για το οποίο ο ρυθμός εκρόφησης απουσία ενζύμου είναι υψηλότερος με πιθανή αιτία την παρεμπόδιση της εκρόφησης, από το ένζυμο και την μερική απορρόφηση εκ νέου του διοξειδίου του άνθρακα.

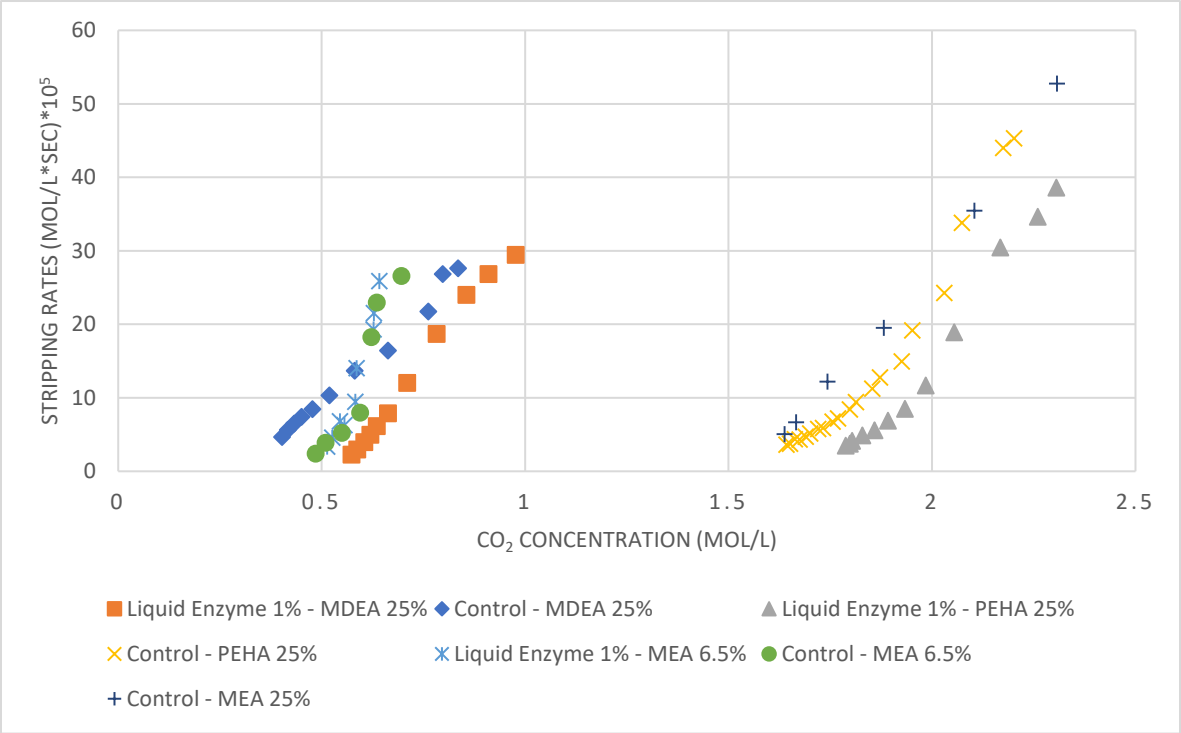


Figure III.12: Ρυθμός εκρόφησης αμινοδιαλυμάτων συναρτήσει της συγκέντρωσης CO_2 στο διάλυμα

Απορρόφηση CO_2 σε πιλοτική μονάδα

Στην πιλοτική μονάδα, εξετάστηκε διάλυμα MDEA, 25% κατά βάρος, χωρίς την προσθήκη ενζύμου. Το δείγμα λαμβάνεται από το μέσο της στήλης απορρόφησης, μετράται το pH και στην συνέχεια, με την μέθοδο $BaCl_2$, υπολογίζεται η συγκέντρωση CO_2 στο διάλυμα.

Εύλογα προκύπτει ότι το pH μεταβάλλεται αντιστρόφως ανάλογα με την συγκέντρωση CO_2, όπως παρουσιάζεται στο διάγραμμα ΙΙΙ.13:

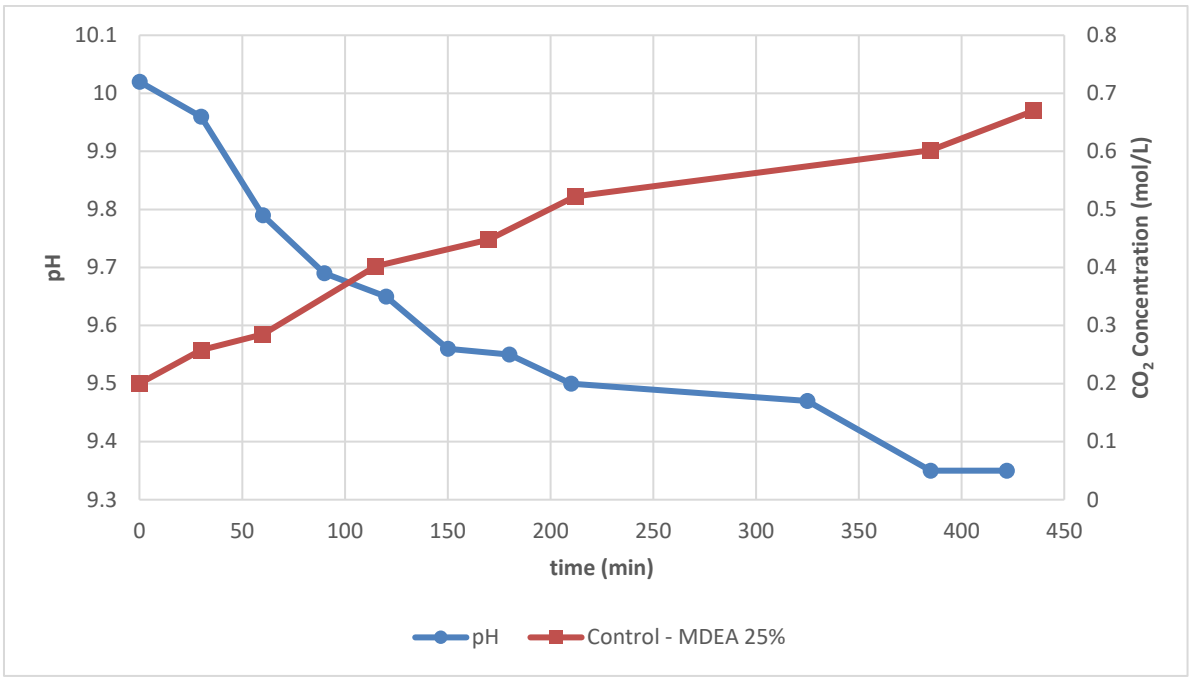


Figure III.13: Μεταβολή συγκέντρωσης CO_2 σε διάλυμα MDEA 25% w/w σε πιλοτική μονάδα απορρόφησης καθώς και η μείωση του pH του διαλύματος

Στην διεργασία εκρόφησης υπολογίστηκε η μεταβολή του CO_2 στο ρεύμα εξόδου με την πάροδο του χρόνου για την οποία παρατηρείται ένα μέγιστο.

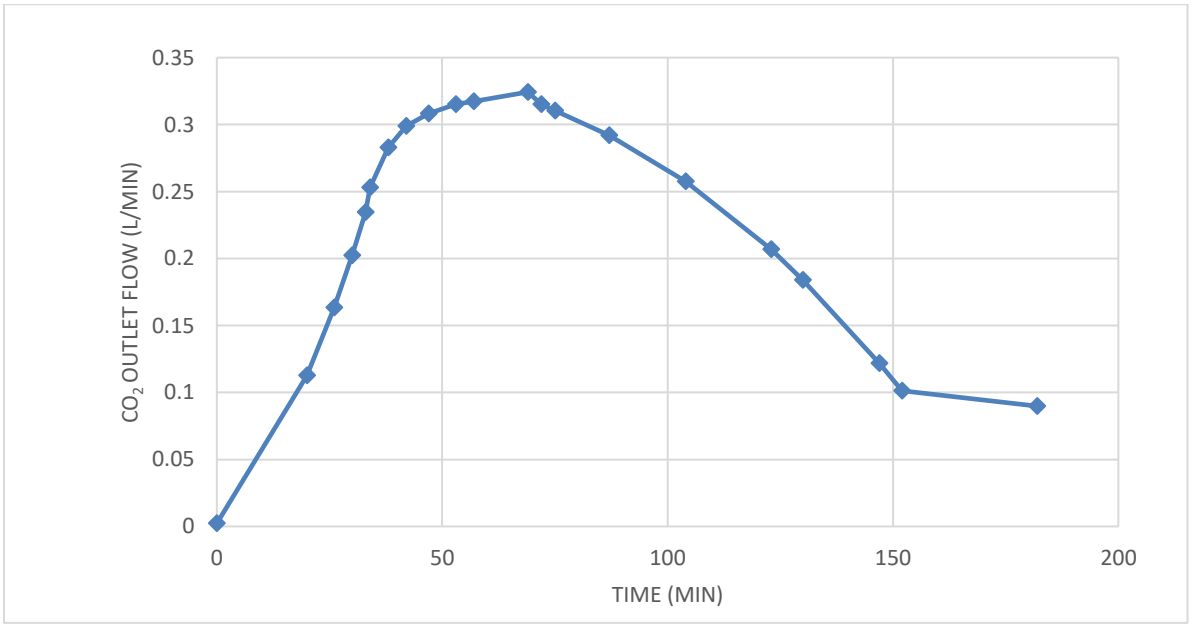


Figure III.14: Συγκέντρωση CO_2 στην έξοδο πιλοτικής μονάδας κατά τη διάρκεια εκρόφησης σε κορεσμένο διάλυμα MDEA 25% w/w

IV. Συμπεράσματα

Η ανθρακική ανυδράση παρουσιάζει σταθερότητα σε υψηλές θερμοκρασίες και σε περιβάλλοντα βασικού pH. Εξετάζοντας τα αποτελέσματα προκύπτει το συμπέρασμα ότι το ένζυμο πράγματι δρα θετικά, για την πλειονότητα των αμινοδιαλυμάτων τα οποία εξετάστηκαν, αυξάνοντας σημαντικά τον ρυθμό απορρόφησης του διοξειδίου του άνθρακα και διατηρώντας σταθερό τον ρυθμό εκρόφησης. Συγκεκριμένα, παρατηρήθηκε αύξηση του ρυθμού απορρόφησης κατά 40% για το MDEA, 9,32% για το PEHA και 8,01% για το MEA. Ωστόσο, με την παρουσία του ενζύμου δεν προκύπτει μεταβολή στην χωρητικότητα των αμινών σε CO_2. Στον εξοπλισμό μεσαίας κλίμακας εξετάστηκαν τα αμινοδιαλύματα διαφορετικών συγκεντρώσεων αλλά και μιγμάτων αυτών. Από τα αμινοδιαλύματα, μέγιστο ρυθμό απορρόφησης παρουσιάζει η MEA, σε συγκέντρωση 25% κατά βάρος, ακολουθούμενη από το ιοντικό υγρό PEHA-προλίνη. Μίγμα των αμινών MDEA-PEHA σε αναλογία 1:1 παρουσιάζει υψηλό ρυθμό απορρόφησης, μεγαλύτερο από το διάλυμα MDEA και παρόμοιο με τον ρυθμό απορρόφησης του PEHA. Μελλοντικά, είναι σημαντική η περαιτέρω διερεύνηση σε μίγματα αμινών και ιοντικών υγρών και η εφαρμογή σε πιλοτική μονάδα.

ABSTRACT

Carbon dioxide (CO_2) is the major contributor of global warming covering more than 60% of greenhouse gases. Major CO_2 production occurs by industries and power plants using fossil fuel. Chemical absorption is an effective and widely used, by industries, method for CO_2 separation and capture due to low equipment cost, high removal efficiency, stable operation conditions, and mature technology background. Main solvent in industry is the alkanolamines with high absorption rates. Depending on the kind of amine (primary, secondary, tertiary) higher rates have been noticed for Monoethanolamine (MEA) following by Pentaethylenehexamine (PEHA) and last Methyldiethanolamine (MDEA). As solvents or in mixtures with alkanolamines, the ionic liquids (PEHA-prolinate) offer stability and lower volatility to the system. The use of biocatalysts (enzymes) into the system has been proven to increase the absorption rates useful. Carbonic anhydrase (CA) is a powerful zinc metalloenzyme that accelerates the transformation of carbon dioxide to bicarbonate ion. A mutant version of the carbonic anhydrase has an activity of the order of 4.5 units/μL enzyme solution or 2.07 units/mg of lyophilized enzyme, which remains quite high when the enzyme appears in high temperatures and basic pH.

In a tiny clave absorption process the maximum capacity in CO_2 differs among the amines with capacity for aqueous solution of MDEA (25% w/w) being 80.7mgCO_2/g of solvent, 122.0 mgCO_2/g of solvent for the PEHA (25% w/w), 109.5 mgCO_2/g of solvent for the MEA (25% w/w) and 82.7 mgCO_2/g of solvent for the ionic liquid PEHA-prolinate (25% w/w). The absorption rates for the alkanolamines and the ionic liquid were calculated 0.75 mgCO_2/g MDEA (25% w/w) /min, 2.79 mgCO_2/g PEHA (25% w/w)/min, 3.12 mgCO_2/g MEA (25% w/w)/min and 1.64 mgCO_2/g PEHA-prolinate (25% w/w)/min. By adding an enzyme solution (1% w/w), an increase of 40%, 9.32%, 8.01% and 4.88% occurred in the absorption rates of MDEA, PEHA, MEA, PEHA-prolinate respectively.

By scaling up the equipment, absorption and stripping rates of alkanolamines and the mixes among them and with ionic liquids were calculated, offering significant results. Specifically, the mix of MDEA-PEHA (1:1 ratio) had an absorption rate higher than the rate of MDEA 25% and close to the rate of the PEHA 25%. The PEHA-prolinate - MDEA (1:1 ratio) did showed a high rate, as well. Experiments were also carried out in a pilot plant for MDEA solution.

1.Introduction

Carbon dioxide (CO_2) has been proven to be a major contributor of global warming covering more than 60% of greenhouse gases. Half of the CO_2 emission is produced by industries and power plants using fossil fuel. The role of CO_2 as a greenhouse gas has resulted in tightening environmental regulations concerning CO_2 emissions to the atmosphere. Thus, achieving a solution for the CO_2 emission reduction is of great interest and a challenge for the researchers. Post-combustion capture and oxy-fuel combustion are the main technologies available. (Cheng-Hsiu Yu, 2012) (A. Masohan, 2009)

Chemical absorption is one of the most effective methods for CO_2 separation and capture. Being of low equipment cost, high removal efficiency, stable operation conditions, and mature technology background, chemical absorption has widely applied to chemical engineering, food industry, and other fields. From the present technologies used, chemical absorption in post combustion capture is the best-known technique, possibly due to the large number of power plants, and the promising results so far. In terms of solvent selection, amines have traditionally been considered as reagents of choice. (A. Masohan, 2009) (A. Bahadori, 2008) (Sanoja A Jayarathna)

According to McCabe, during the absorption of gases a soluble vapor is absorbed by the mixture with an inert gas by means of a liquid in which the dissolved gaseous substance is more or less soluble. The dissolved substance is recovered from the liquid by distillation and the absorbent liquid can be discarded or reused. Sometimes the solute is removed from the liquid by bringing the liquid into contact with an inert gas. This process, which is the inverse of gas absorption, is called desorption or stripping. (W. L. McCabe, 6th Edition)

1.1 Absorption

In the absorption process two phenomena may take place, the physical absorption and the chemical absorption. The operation of physical absorption is based on Henry's Law. CO_2 is absorbed under high pressure and a low temperature and desorbed at reduced pressure and increased temperature. Chemical absorption is based on the solubility difference between CO_2 and the other gas components in mixed gases. It can be classified into nonrecycling processes and recycling processes. For the former processes, the rich CO_2 solution cannot be regenerated. While for the latter processes, the absorbent can be recovered by the cycle of absorption and regeneration. (Lars Erik Oi, 2013) (Xiao Luo, 2017)

1.1.1Mechanism of Chemical Absorption

During the chemical absorption process, the CO_2 solubility depends on the physical solubility in the absorbent, the chemical reaction equilibrium constant, the chemical equivalent ratio, and other factors. In addition, in cases where the chemical absorbent solution is a strong or weak electrolyte, dilute solution theory does not fit. Solubility of gases in the chemical absorbent is characterized by a uniform solubility increase with pressure. The higher the pressure, the lower the solubility enhancement is. In that case, the relationship between partial pressure and gas solubility is more complex than that of physical absorption. (Cheng-Hsiu Yu, 2012) (S. Santos, 2016)

The difference between chemical absorption and physical absorption is whether CO_2 reacts with the absorbent during the absorption process.

1.1.2 Henrys Law

The liquid-gas solutions are systems of two components (e.g.: liquid solvent and gaseous solute). In the absorption process, the solubility of the gas to be absorbed in the liquid is an important factor and depends on the temperature and the pressure of the system. Therefore, there is a gas-liquid equilibrium curve, connecting the solute gas equilibrium concentrations to each temperature, divided into the gaseous and liquid phase (D. K. Asimakopoulos, 2012, pp. 448-450).

The equilibrium curve can be constructed according to the following equation, by setting equal to 1 both the coefficient of the soluble gaseous component at pure gaseous state, at operating temperature and pressure, and the coefficient of activity of the dissolved gas:

$$H = \frac{y}{x} \cdot P \quad \text{(eq. 1.1)}$$

Where: H = equilibrium constant depending on the nature of the gas to be absorbed and the temperature of the absorber liquid

P = total absolute pressure of the gaseous mixture

x = molar fraction of the solute (gas) in gaseous phase

y = molar fraction of the solute (gas) in the liquid phase

According to Dalton's Law for diluted solutions and ideal gases, the total pressure of the system is equal to the sum of the partial pressures of the individual gases.

$$y^* P = p \quad \text{(eq. 1.2)}$$

where: p, partial pressure of the gas to be absorbed

Therefore:

$$H = \frac{p}{x} \quad \text{(eq. 1.3)}$$

Consequently, according to Henry's Law the molar fraction of a gas dissolved into a liquid, at a given temperature, is proportional to its partial pressure.

The Henry's constant (H) depends on the temperature, the nature of the solvent and the nature of the absorbed gas.

1.1.3 Fick's Law

If we consider two different phases (gas-liquid) in mutual contact, containing a soluble gas component in both phases, but distributed in different concentrations, this component passes from one phase to the other to reach equilibrium of concentrations.

To perform this passage, the gas to be absorbed meets two resistances, the first in spreading the higher concentration from the phase center to the interphase zone, the second is the one it meets when passing from the interphase zone to the core of the second phase (Figure 1.1).

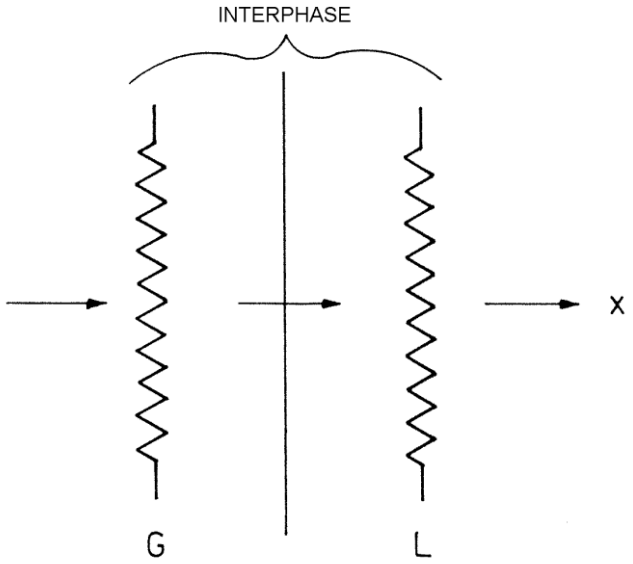


Figure 1.1: An example of the resistances that the gas meets during absorption. The first resistance (G) is the spreading of the higher concentration from the phase center to the interphase zone. The second resistance (L) is passing from the interphase zone to the center of the liquid phase.

Supposing the two phases are stagnant or move with laminar motion which is orthogonal to the migration direction, the absorption speed is defined by Fick's Law, expressing the quantity of material crossing the surface unit in the time unit (D. K. Asimakopoulos, 2012, pp. 468-470):

$$G_S = - D \frac{dc}{dx} \text{ (eq. 1.4)}$$

where: G_S = mass of gas to be absorbed (in gr/cm^2 s or in kg/m^2 h) which in the time unit spreads through the surface unit along the direction n

D = coefficient of diffusion in cm^2/s or m^2/h, these values are reported in Tab.

dc = gradient of concentration in gr/cm^3 or kg/m^3 reporting the infinitesimal concentration difference between points x and x + x along the migration direction.

Developing equation 1.4 and reporting it into a graph on the x and y coordinates, you obtain the curve shown in Figure 1.2.

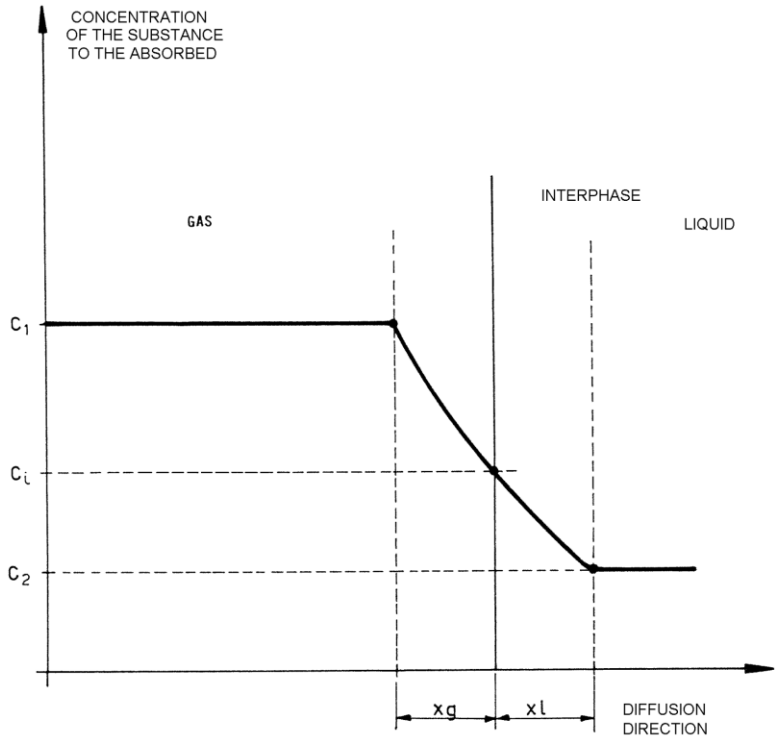


Figure 1.2: The Fick's Law showing the quantity of material crossing the surface unit in the time unit

This curve can be applied to stagnant phases and to laminar motions with an orthogonal diffusion direction in respect to the diffusion direction. But, if the phases are continuously mixing - i.e. they are in continuous motion - the irregular motion of the fluid particles in each phase facilitates the matter transport, this means the resistance to diffusion drops, therefore, Fick's law transforms into:

$$Gs = -(D + \varepsilon\alpha)\frac{dc}{dx} \quad \text{(eq. 1.5)}$$

At this point, we must add a consideration: the effect of the turbulence in the two phases is the continuous mixing of the mass, and, as the speed and direction of the single particles change, it follows that, considering any point of the mass, the concentration gradient is very small (dc / dx) and so negligible. Consequently, the behavior of the concentrations of each single phase is constant and can be graphically represented by a straight line, but in

proximity to the interphase surface, which can be considered an actual wall limiting the phases, there are two stagnant films, so εα is null and:

$$\frac{dc}{dx} < 0 \text{ (eq. 1.5)}$$

Then, we can say that in the core of matter (continuously mixing) the resistance to diffusion is null and is localized in the extreme layers of both phases near the interphysical surface and localized in the liquid film and in the gaseous film.

Applying Fick's law to the two films:

$$Gs = \frac{Dg}{xg} \cdot (C_1 - C_i) \cdot \frac{D_1}{x_1} \cdot (C_i - C_2) \text{ (eq. 1.6)}$$

where: D_g = coefficient of diffusion of the component diffusing in the gas

D_1 = coefficient of diffusion of the component diffusing in the liquid

C_1 = concentration in the gaseous phase

C_i = concentration in the interface

C_2 = concentration in the liquid

x_g = gaseous film thickness

x_1 = liquid film thickness

Equation (1.6) cannot be solved mathematically, as xg and x1 are complex functions of the Reynolds Number, temperature and pressure and also of the equipment geometry which is used for the process.

The values Dg/xg and D1/x1 are determined experimentally, through the dimensional analysis and the correlation of the experimental data, so that:

$$\frac{Dg}{xg} = hg \quad \textit{(eq.1.7)} \qquad\qquad \frac{D1}{x1} = h1 \textit{ (eq. 1.8)}$$

The coefficients hg for the gas and h1 for the liquid are called coefficients of diffuse transmission.

It is better to consider the fact that the motive power (i.e. the power used to pass between the two phases) can be expressed in different ways which corresponds to a different transfer - numerically as well as dimensionally.

Among the values of tabulated h there are the following relations:

* for the gas:

$$h_g = \frac{h'_g}{P} = \frac{h'_g}{RT} \text{ (eq. 1.9)}$$

where: P = total pressure of the gaseous mixture

T = absolute temperature

* for the liquid:

$$h_1 = \frac{h'_1}{c} \text{ (eq. 1.10)}$$

where: c = total molar concentration, i.e. the moles of gas + liquid contained in a liter of solution.

1.2 Desorption – Stripping

1.2.1 Regeneration Process

In industry, an effective and less energy-consumed regeneration of the CO_2 captured adsorbents is definitely needed to develop. Currently the regeneration techniques include pressure swing adsorption (PSA), vacuum swing adsorption (VSA), temperature swing adsorption (TSA), electric swing adsorption (ESA), the increase of temperature by conducting electricity through the conductive adsorbents, pressure and temperature hybrid process (PTSA) and washing. (Cheng-Hsiu Yu, 2012)

In PSA, adsorption is typically performed at pressures higher than atmospheric pressure, while desorption is performed at atmospheric pressure. In VSA, adsorption operates at atmospheric pressure and near-room temperature and desorption operates at lower

pressures. Both PSA and VSA are performed by altering the pressures. Generally, the adsorbents with high adsorption capacity and high selectivity toward CO_2 are preferred.

In order to decide whether a regeneration process is successful and be able to compare it to other processes some performance parameters has been proposed such as CO_2 purity, CO_2 recovery, and power consumption. Specifically, the power consumption and CO_2 capture cost are significantly influenced by adsorbent type, process configuration, and operating parameters such as feed gas pressure, vacuum pressure, temperature, and extents of purge. In TSA, the regeneration is achieved by hot air or steam. The regeneration time is in general longer than PSA. (Cheng-Hsiu Yu, 2012)

Desorption by heating with water vapor (TSA) was not very favorable, due to the high vapor consumption needed for heating the column. Moreover, the time needed for regeneration was much longer than the adsorption step since the thermal inertia resulted in long heating and cooling times. Therefore, the total number of columns in a regeneration mode for each column would be largely increased. (B. Lv, 2015)

The recovery depends on desorption temperature and purge flow rate. In ESA, heat is generated by Joule effect via electric current passing through the adsorbents. The instant heating route can effectively deliver heat to adsorbents without heating the additional media, thus ESA offers several advantages including less heat demanded, fast heating rate, better desorption kinetics and dynamics and independent control of gas and heat flow rates as compared with PSA and TSA.

In recent years, one kind of researches is to find absorbents with high absorption rate and low energy requirement of CO_2 desorption. Another one is to optimize the parameters during the process of CO_2 absorption and desorption, that is the effect of temperature, CO_2 loading on CO_2 absorption, on the mass transfer coefficients of CO_2 absorption in aqueous ammonia solution, the effect of flow and gas concentration on CO_2 and the pH. (Du, 2017)

1.3 Chemistry of the process

1.3.1 Carbonate system

In nature the water (lakes, rivers, sea) operates as CO_2 absorption solution. Water tends to be in equilibrium with the partial pressure of CO_2 in the atmosphere. The CO_2 is absorbed in the water and then dissolves and reacts with it to carbonic acid. Most dissolved CO_2 occurs as aqueous CO_2 rather than carbonic acid. The carbonic acid is in a balanced equation to a bicarbonate and a proton. The bicarbonate is also in a balanced equation to a carbonate and a proton closing the carbonate system as it is shown in figure 1.3. (Cheng-Hsiu Yu, 2012)

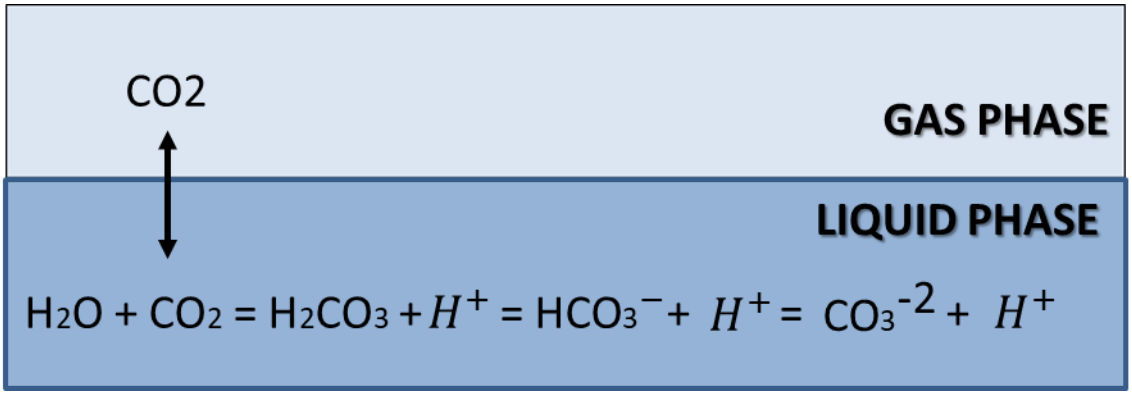


Figure 1.3: The carbonate system

1.3.2 General about amines and alkanolamines

1.3.2.1 Absorption reactions of alkanolamines with CO_2

A general amine has the formula NR1R2R3 where R1, R2 and R3 are organic groups or hydrogen directly bonded to a central nitrogen atom. An amine with only one organic group directly bonded to nitrogen, is a primary amine, with two organic groups it is a secondary amine and with three it is a tertiary amine. If an organic group contains an OH-group, the amine is called an alkanolamine. Alkanolamines are widely used as the absorbents for CO_2 capture. The reactivity of amines to CO_2 follows the order primary, secondary and ternary amines, for example, the reaction constants with CO_2 are 7,000, 1,200 and 3.5 m3 /s/kmol for MEA, DEA and MDEA at 25°C, respectively. On the contrary, the CO_2 loading capacity for ternary amine is 1.0 mole of CO_2 per mole of amine, higher than those of primary and

secondary amines where the CO_2 loading capacity lies between 0.5–1.0 mole of CO_2 per mole of amine, as can be seen from the following equations (Cheng-Hsiu Yu, 2012):

$$RR'NH + CO_2 \leftrightarrow RR'NH + COO^- \text{ (Zwitterion)}$$

$$RR'\ NH^+\ COO^- + RR'NH \leftrightarrow RR'NCOO^- \text{ (Carbamate)} + RR'NH_2^+$$

The overall reaction is:

$$2RR'NH + CO_2 \leftrightarrow RR'NCOO^- + RR'NH_2^+$$

$$RR'NCOO^- + H_2O \leftrightarrow RR'NH + HCO_3^-$$

The reaction of primary and secondary amines with CO_2 is to form zwitterion first and then to form carbamate. The reaction of a tertiary amine with CO_2 is to form bicarbonate but not to form carbamate.

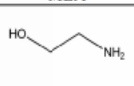
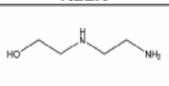
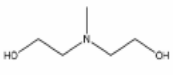
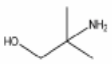
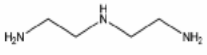
Property	MEA	AEEA	PZ	MDEA	NaOH	AMP	DETA
Molecular structure							
MW (g/mol)	61.08	104.15	86.14	119.16	40	89.14	103.17
Density (293K) (g/cm^3)	1.012	1.029	1.1	1.038	1.515	0.934	0.955
Boiling point (K)	443	513	420	243	1390	438	207
Vapor pressure (293K) (kPa)	0.0085	0.00015	0.1066	0.0013	0.4	0.1347	0.02
Vapor pressure (393K) (kPa)	15.9	0.969	41.66				
Solubility (293K)	Freely soluble	Freely soluble	14 wt%	Freely soluble	Freely soluble	Freely soluble	Freely soluble
Pseudo first order rate constant at 298 K (m^3/kmol/s)	7,000 Hikita *et al.*, 1979	12,100 Mamun *et al.*, 2007	53,700 Bishnoi and Rochelle, 2000	3.5 Bishnoi and Rochelle, 2002		681 Xiao *et al.*, 2000	49,740 Hartono *et al.*, 2009
Activation energy (kJ/mol)	46.7 Mandal *et al.*, 2001	N/A	35 Cullinane and Rochelle, 2004	44.3 Pani *et al.*, 1997		41.7 Alper, 1990	
CO_2 Absorption capacity (mol of CO_2/mol of absorbent)	0.5	1.0	1.0	1.0	0.5	1.0	1.0

Figure 1.4: Table of properties for different amines (Cheng-Hsiu Yu, 2012)

Because of various properties and advantages of various amines, mixed amines have been proposed to enhance CO_2 capture efficiency and to reduce regeneration cost. In addition to the primary, secondary and tertiary amines, the steric hindrance amines such as 2-amino-2-methyl-1-propanol (AMP) were also proposed to use. This is due to the fact that the steric character reduces the stability of the formed carbamate, thus carbamate can undergo

hydrolysis to form bicarbonate and simultaneously, releases free amine molecules for further reaction with CO_2 and consequently enhance the CO_2 equilibrium loading capacity to 1.0 mol of CO_2 per mol of amine, as high as that of ternary amine. (Xiao Luo, 2017)

1.3.2.2 Alkanolamine's degradation and corrosion rates

The degradation of alkanolamine is an important issue in chemical absorption processes because it causes economic, operational, and environmental problems. For a commonly used absorbent, for example, MEA, the degradation would cause the replacement of ~2.2 kg MEA for capturing one tonne of CO_2, leading to an increase of operation cost. Degradation can generally be classified into three types, thermal degradation, carbamate polymerization, and oxidative degradation. (N. Hatcher, 2014)

Thermal degradation requires the operation at high temperatures, generally above 200°C. This kind of degradation does not occur for dealing with the power plant exhausted gases because the operation temperature in thermal regeneration is not as high. (N. Hatcher, 2014)

Oxidative degradation is mainly resulted from the dissolved oxygen (DO) in absorbent. Hence this type of degradation often occurs in CO_2 capture from the flue gases containing high O_2 content. To reduce the dissolved oxygen in absorbent, four methods including the addition of O_2 scavenger, reaction inhibitor, chelating agents, and strong stable salts have been proposed. Three additives, Inhibitor A (an inorganic compound), Na_2SO_3, and formaldehyde have been suggested. (Cheng-Hsiu Yu, 2012)

Carbamate polymerization requires the presence of amine at high temperatures so that it typically occurs in the stripping during the thermal regeneration.

Corrosion rates are affected by the nature of the corrosive agent, temperature, fluid velocity, the presence of solids, and the metallurgy involved. In an amine water system, the formatted acids are the corrosive agents and not the amine itself. In post combustion the main gases that can cause corrosion are the H_2S and the CO_2. The corrosive action of H_2S is inherently different from that of CO_2 as H_2S can form a protective iron sulphide layer on the metal surface. On the other hand, iron carbonate forms a more fragile layer, so it offers

much less protection. At the same time high fluid velocities physically increase corrosion rates as it does a higher temperature. (Cheng-Hsiu Yu, 2012) (F. Closmann, 2011)

As far as the corrosive agents themselves are concerned, the important parameter is the chemical activity of the dissolved acid gas species responsible for corrosion. The activity (vs concentration) changes with the amine type, amine concentration, acid gas loadings, the concentrations and temperature. However, the critical parameter for the corrosion rates is the activity of the corrosive species and in less point the type of amine. The chemical species of interest are: bisulphite ion (HS^-), free physically dissolved H_2S, bicarbonate ion (HCO_3^-), and free physically dissolved CO_2, all of which are oxidising agents. Sulphide (S^{2-}) and carbonate (CO_3^{2-}) ions are also present; however, they themselves are final reaction products and are unable to provide the hydrogen ion necessary for the oxidation of iron. Molecular hydrogen sulphide and carbon dioxide react with iron only in the presence of water. The final distribution of molecular and ionic species is found by solving the equations of chemical reaction equilibria, atom balances, and a charge balance. The resulting set of species concentrations is termed the solution's speciation. (Cheng-Hsiu Yu, 2012)

In the simplest stoichiometric forms, the basic corrosion reactions of iron with H_2S species are:

$$H_2S(aq) + Fe(s) \rightarrow FeS(s) + H_2\,(g)$$

$$2HS^-\,(aq) + Fe(s) \rightarrow FeS(s) + H_2\,(g) + S^{2-}(aq)$$

And with the CO_2 species are:

$$CO_2\,(aq) + Fe(s) + H_2O \rightarrow FeCO_3\,(s) + H_2\,(g)$$

$$2HCO_3^-\,(aq) + 2Fe(s) \rightarrow 2FeCO_3\,(s) + H_2\,(g)$$

The oxidation reaction with hydrogen sulphide is faster than the reaction with bisulphide; however, the alkalinity of the amine (and ammonia) solutions means that the dissolved H_2S is predominantly in the bisulphide form, with very little remaining as free molecular hydrogen sulphide. The same is happening for the dissolved carbon dioxide. The concentrations of free H_2S and CO_2 are pH dependent and pH is a function of amine strength, total dissolved acid gas, temperature, and to a lesser extent heat stable salts

concentration. However, heat stable salt species and their concentrations do affect the speciation of the solution, especially in lean solvents. (N. Hatcher, 2014)

What limits the corrosion reactions is primarily the amount of bare, unreacted iron that the passivating film leaves available at the metal surface. A secondary factor is the concentrations of dissolved reactant gases, H_2S and CO_2. Thus, H_2S, HS^-, CO_2 and HCO_3^- all react with unprotected iron. To control the concentration of dissolved gas in their various forms, a rich amine acid gas loading of upper limit of 0.4 to 0.5 moles of total acid gas per mole of molecular amine, can be used. (N. Hatcher, 2014)

In a new amine unit or a fresh cleaned one, right after the introduction of the gas (H_2S or CO_2) the corrosion rate climbs exponentially. Eventually, the corrosion rate levels off as the iron sulphide/carbonate layer increasingly occludes (passivation), until the iron sulphide/carbonate layer is established when the system settles down to a nominal residual corrosion rate. During these three phases of passivation, the solution first becomes nearly black, and then it changes to dark green, lighter green, and finally slightly amber. Chelated iron (which results from complexation) scatters light and turns otherwise contaminant-free solution to amber. The coloration depends on the size of the iron sulphide/carbonate particles. It must be marked though that the precipitated layer of iron carbonate is very fragile, so it is unlikely to adhere as tenaciously to metal surfaces as iron sulphide does. Main corrosion begins primarily with protecting the iron sulphide film on the metal. Fluid velocity creates sheer stress on solid surfaces — the shear stress can be enough to rip off the imperfect iron sulphide layer, and even a low velocity seems very likely to dislodge and remove iron carbonate deposits. When this happens, fresh iron is exposed and corrosion increases in the region of high shear stress. Subsequently, the dislodged iron sulphide and iron carbonate particles can act as scouring agents and increase corrosion in other areas of the plant via that mechanism. For these reasons, the gas treating industry has generally adopted velocity limits for carbon steel piping in amine service. Even a little flashing can greatly increase the velocity in a pipe, and the high velocity flow from the passing biphase scours the iron sulphide layer. This results in drastically shorter piping life. Although less accurately than for single-phase flows, it is still possible to provide reasonable estimates of corrosion rates in two-phase flows, and this is done in the present model. Heat stable salts affect corrosion rates by altering solution speciation, and they chemically exacerbate

corrosion by chelating iron and destroying the passivating iron sulphide layer (N. Hatcher, 2014):

$$FeS + 7\ H_2 0 \leftrightarrow Fe(H_2O)_6{}^{+2} + HS^- + OH^-$$

$$Fe(H_2\ 0)_6{}^{+2} + n\ HCOO^- \leftrightarrow + Fe(n\ HCOO^-)^{(2-n)} + (6 - n)H_2O$$

1.3.3 N- Methyldiethanolamine (MDEA)

MDEA, $H_3CN(CH_2CH_2OH)_2$, is a tertiary alkanolamine in which the nitrogen is bonded to two ethanol groups and a methyl group. Tertiary alkanolamines are differentiated from the primary and secondary ones in their reaction with CO_2 resulting in a slower reaction compared with that of the other amine types, because the carbamate formation is not possible. It has been accepted that the reaction order of the chemical absorption process of carbon dioxide in aqueous solutions of MDEA is one with respect to the carbon dioxide and the alkanolamine, giving an overall reaction order of two. The reaction might be described with a kind of base catalysis of the CO_2 hydration. This catalytic effect is based on the formation of a hydrogen bond between the amine and water. This weakens the bond between hydroxyl group (-OH) and hydrogen, and then increases the water nucleophilic reactivity toward carbon dioxide. The mechanism followed is given as (F. Camacho, 2008) (P.J.G. Huttenhuis, 2007) (A. Bahadori, 2008):

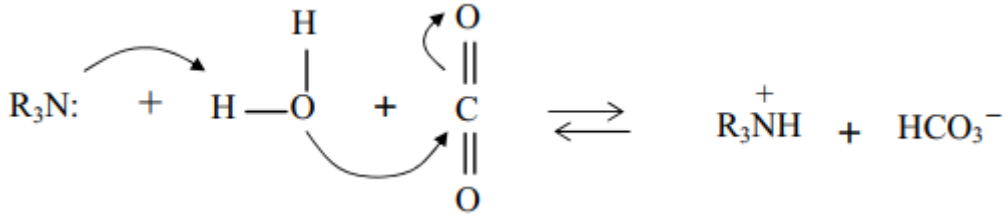


Figure 1.5: MDEA reaction with CO_2 in aqueous solution

Considering the process as a two-stage reaction:

$$H_2O + R_3N \leftrightarrow OH^- + R_3NH^+ \text{ (instantaneous)}$$

$$OH^- + CO_2 \leftrightarrow HCO_3^- \text{ (slow)}$$

The overall mechanism also included the reactions of the carbonic acid formation and the bicarbonate ion formation.

Carbonic acid formation: $CO_2 + H_2O \leftrightarrow H_2CO_3$

Bicarbonate formation: $CO_2 + OH^- \leftrightarrow HCO_3^-$

On an industrial scale, MDEA is an alkanolamine of great interest in acidic-gas absorption and thus presents less corrosive effects than the primary or secondary alkanolamines. Furthermore, because of the instantaneous reaction rate of the H_2S on the CO_2, when these are present in a gas current and their separation is sought for later use of the H_2S, or when the CO_2 is not necessary or economically feasible. On the other hand, MDEA has some advantages compared to others alkanolamines used as absorbents such as monoethanolamine, because MDEA presents higher equilibrium capacity conversion than does MEA, which is limited by the stoichiometry. Similarly, due to the reactive characteristics, the CO_2-MDEA process takes place with a lower reaction enthalpy than for aqueous solutions of primary or secondary alkanolamines, and this is causing its use to increase. Also, they require less regeneration energy and are resistant to the thermal and chemical degradation. (P.J.G. Huttenhuis, 2007) (F. Camacho, 2008)

1.3.4 Monoethanolamine (MEA)

Due to its high reactivity with CO_2, monoethanolamine has been used in industrial processes to capture CO_2 for many years. In the past few decades, lots of experimental and theoretical works have been carried out to investigate the reaction mechanism of CO_2 absorption into MEA solution. Nevertheless, there is still a controversy regarding to the details of the reaction mechanism.

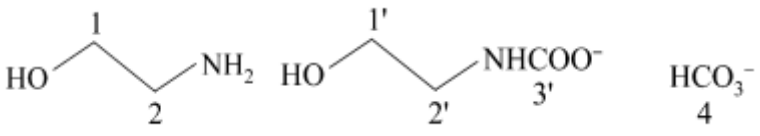


Figure 1.6:Molecular structure and type of carbon nuclei in MEA-CO_2 system.

There were three reaction mechanisms proposed for the reaction between CO_2 and MEA. However, the zwitterion mechanism is the most commonly accepted. This mechanism

suggested that primary and secondary alkanolamines were first reacted with CO_2 to form zwitterions and then the intermediate was instantaneously neutralized by the base (such as amine, OH−, or H_2O) to form carbamate (B. Lv, 2015).

$$CO_2 + RNH_2 \rightleftharpoons RNH_2^+COO^-$$

$$RNH_2^+COO^- + RNH_2 \rightleftharpoons RNHCOO^- + RNH_3^+$$

Meanwhile, it is also widely used to explain the reaction of CO_2 absorption into other solvents, for example, mixed amine and functionalized ionic liquids.

1.3.5 Pentaethylenehexamine (PEHA)

Pentaethylenehexamine has two primary and four secondary amine groups in the structure, as it is shown in figure 1.7. Due to this high amine group content per unit mass, its high thermal stability and its low toxicity it can be a good candidate for the post combustion CO_2 capture. (Q. Ye, 2015)

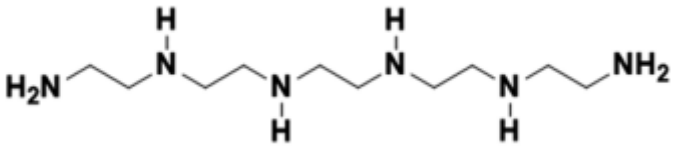


Figure 1.7: The chemical structure of the PEH amine

Nevertheless, there is little detailed information available on the CO_2 capture performance of aqueous PEHA.

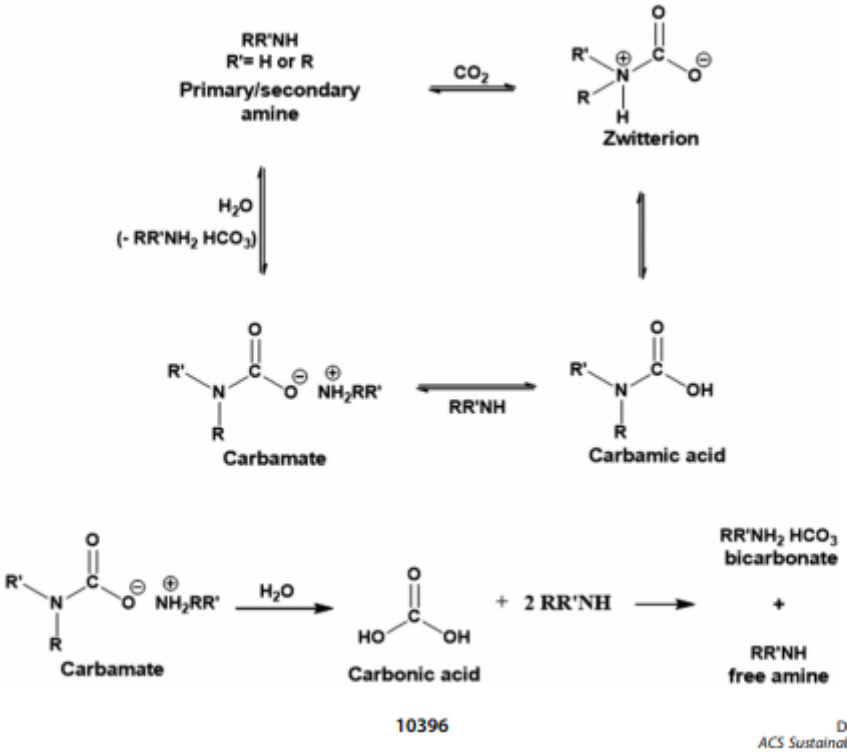


Figure 1.8: Plausible Reaction Mechanism of an unhindered primary/secondary amine with CO_2 in aqueous media

1.3.6 Ionic liquids

Ionic liquids (ILs) are organic salts that form stable liquid below 100 ◦C or even at room temperature (room temperature ionic liquids, RTILs). For the removal of acidic gases, compared with alkanolamines, RTILs have negligible volatility and significant thermal stability that avoids loss of absorbent. By introducing functional groups that could enhance the CO_2 absorption into the anion or cation, functionalized ionic liquids are expected to have significant performance in the uptake of CO_2. The IL could be regenerated upon heating (80–100 ◦C) for several hours under vacuum. (Z. Feng, 2010)

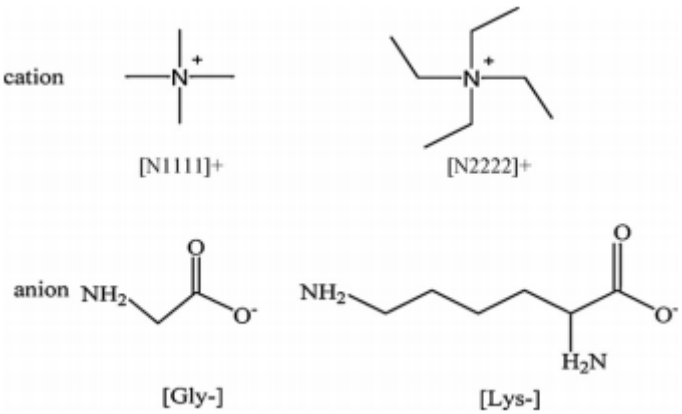


Figure 1.9: Cation and onion of amino acid ionic liquids

The disadvantages of all ILs, particularly the functionalized ILs, are the high viscosity and cost, which seriously hinder their application in the separation of sour gases. In the light of methods and theories of the mixed amines, mixing functionalized ILs with MDEA solution is a good way to improve the usage of ILs in the absorption of CO_2. In general, the amino acid ILs with very high solubility in water, lead to a very high concentration of IL. (Z. Feng, 2010)

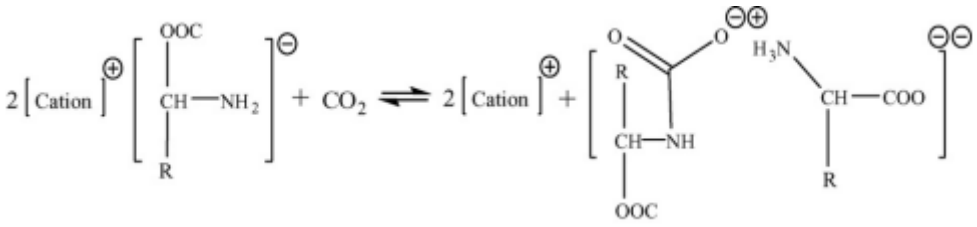


Figure 1.10: Ionic liquid reaction with CO_2

Recently, ionic liquid has attracted widespread attention owing to its unique properties such as very low vapor pressure, good thermal stability, high polarity, and non-toxicity. Ionic liquids have been extensive used as the solvents for catalysis and synthesis. For CO_2 capture, ionic liquids can be applied to absorb CO_2 by either physical absorption or chemical absorption. For physical absorption, the factors influencing CO_2 solubility in ionic liquids include free volume and size of ionic liquids as well as cation and anion. In general, anion has more influence on CO_2 solubility than cation. For use of ionic liquids in chemical absorption, their structure containing amino-function group that can react with CO_2 can be selected. This kind of ionic liquid is called task-specific ionic liquid (TSIL), because ionic liquid is synthesized with the desired properties. The CO_2 absorption capacity of TSIL was three times higher than that of the ionic liquid in physical absorption. However, there exists a limitation of TSIL for

CO_2 absorption that is high viscosity possessed by TSIL which is due to the formation of hydrogen bond between cation and anion. (Cheng-Hsiu Yu, 2012)

1.4 Scale up effects

From the existing capture technologies, the mature technology is, currently, chemical absorption using aqueous amine solutions. Chemical absorption is a well-known technology, and it has been widely deployed on a large scale across several industries. In terms of solvent selection, amines have traditionally been considered as reagents of choice, whereas a primary alkanolamine, monoethanolamine (MEA), is typically considered the benchmark to which alternative solvents are compared. Other compounds that are often considered are piperazine (PZ), diethanolamine (DEA), and methyldiethanolamine (MDEA). One major setback in this process is that MEA has the limitation of a maximum CO_2 loading capacity, based on stoichiometry of approximately 0.5 mol CO_2/mol amine—unlike tertiary amines such as MDEA, which has an equilibrium CO_2 loading capacity nearly of 1.0 mol CO_2/mol amine. The mechanism involved in the absorption of CO_2 by aqueous solutions of tertiary amines such as MDEA is somewhat different to those of primary and secondary amines, as they do not react directly with CO_2. In fact, they act as a base, catalyzing the hydration of CO_2. Thus, the reaction of interest in aqueous solutions of tertiary amines is:

$$CO_2 + H_2O + R1R2R3N = R1R2R3NH^+ + HCO_3^-.$$

Technologically, a typical chemical absorption process consists of an absorber and a stripper, in which the absorbent is thermally regenerated. In a chemical absorption process, as shown in figure 1.11, the flue gas containing CO_2 enters a packed bed absorber from the bottom and contacts in counter-current with a CO_2-lean absorbent. After absorption, the CO_2-rich absorbent flows into a stripper for thermal regeneration. After regeneration, the CO_2-lean absorbent is pumped back to the absorber as a cyclic mode. The pure CO_2 released from the stripper is then compressed for the subsequent transportation and storage (S. Santos, 2016).

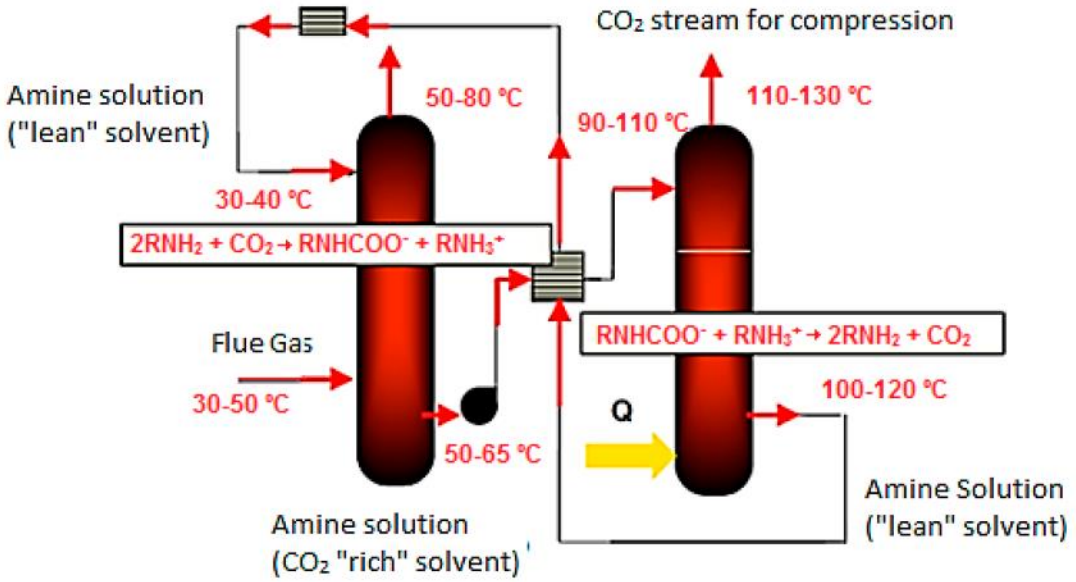


Figure 1.11: Process plan for CO_2 absorption using amines with the respective temperatures and reactions

The main challenge for scaling-up is to progress from the experimental demonstration of the concept at an increasing scale and under realistic conditions, simultaneously validating the expected benefits and overcoming the obstacles that may appear in the path towards large-scale demonstration. In particular, the actual need to perform scale-up for CO_2 absorption plants is considered critical, as the existing ones are not enough to deal with the current huge amount of CO_2 emissions to the atmosphere. This requirement is related to both the CO_2 flow rate and the loading capacity of solutions being used to absorb it. (S. Santos, 2016) (Sanoja A Jayarathna)

When analyzing results from different scales of equipment, some differences occur that may be a result from the process scale-up. Scaling up any process can result in several difficulties for chemical engineers due to the fact that the relative importance of process variables affecting performance considerably increases. In fact, process variables, such as CO_2 injection point in the absorption column, reactant flow, contact time between the reactants throughout the absorption column, temperature and pressure control, and maintenance, have a marked influence on the performance of the units. These variables are somewhat easier to control at laboratory scale and at a small pilot unit, but its significance becomes considerably relevant for larger scales.

These scale-up effects are, in fact, quite difficult to quantify but tend to considerably affect the performance of the absorption process, particularly when progressing for even larger scales, such as industrial plants. However, a way to decrease the significance of the inconvenient scale-up effects will be to complement the scaling-up process by additionally including the use of simulation tools in order to help in the selection of the best operating conditions for the process, contributing to high production yields and thus a more profitable operation. In fact, the use of steady-state or dynamic models for simulation, taking into account precise geometry factors of the columns, as well as operating factors such as the flow rate of each phase, could help to reduce the inaccuracy of the estimation process. Particular care should be taken in the scale-up of chemical processes involving unit operations such as absorption and stripping, which are, in fact, affected by a multitude of operational factors, apart from dimensional ones. (S. Santos, 2016)

1.5 Carbonic Anhydrase

Although the alkanolamines are the most preferably solvents in CO_2 absorption/stripping processes, the cost is still quite high especially when it comes to the energy demands in the regeneration process. In tertiary amines the regeneration energy is significantly lower than the regeneration energy of primary and secondary amines. However, an ideal solution would be a combination of fast absorption and low regeneration energy- such as activated tertiary amine solutions. At present, the addition of small amounts of a (fast reacting) activator to such a solution is finding more and more application in the bulk removal of carbon dioxide. Well-known activators are amines, such as piperazine. Other chemical additives can also be employed such as hypochlorite. Another approach is to utilize a biocatalyst, the enzyme carbonic anhydrase. (Nathalie J.M.C. Penders-van Elka, 2012)

Carbonic anhydrase (CA) is a powerful zinc metalloenzyme that accelerates the transformation of carbon dioxide to bicarbonate ion. CA appears among others in the blood of humans and other mammals, and facilitates the transfer of CO_2 during respiration. Genetic modification of this enzyme makes it possible to use it in combination with aqueous alkanolamine solutions within an industrial environment, like flue gas treatment. (Nathalie J.M.C. Penders-van Elka, 2012) (Cecilia Forsman, 1988) (Oscar Alvizoa, 2014)

Carbonic anhydrase, a very efficient catalyst that enhances the reversible reaction of CO_2 to HCO_3^-, was first identified in 1933 in red blood cells. (Nathalie J.M.C. Penders-van Elka, 2012)

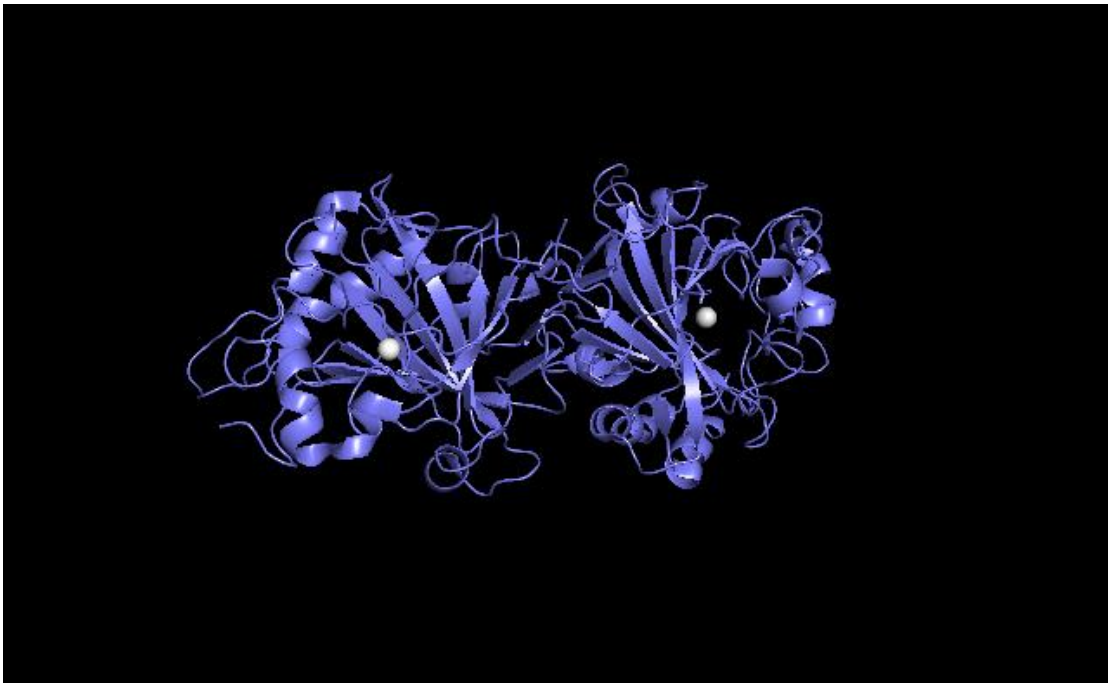


Figure 1.12: Carbonic Anhydrase isozyme XIII structure based on the β-pleated sheets and the α-helix and the zinc active center

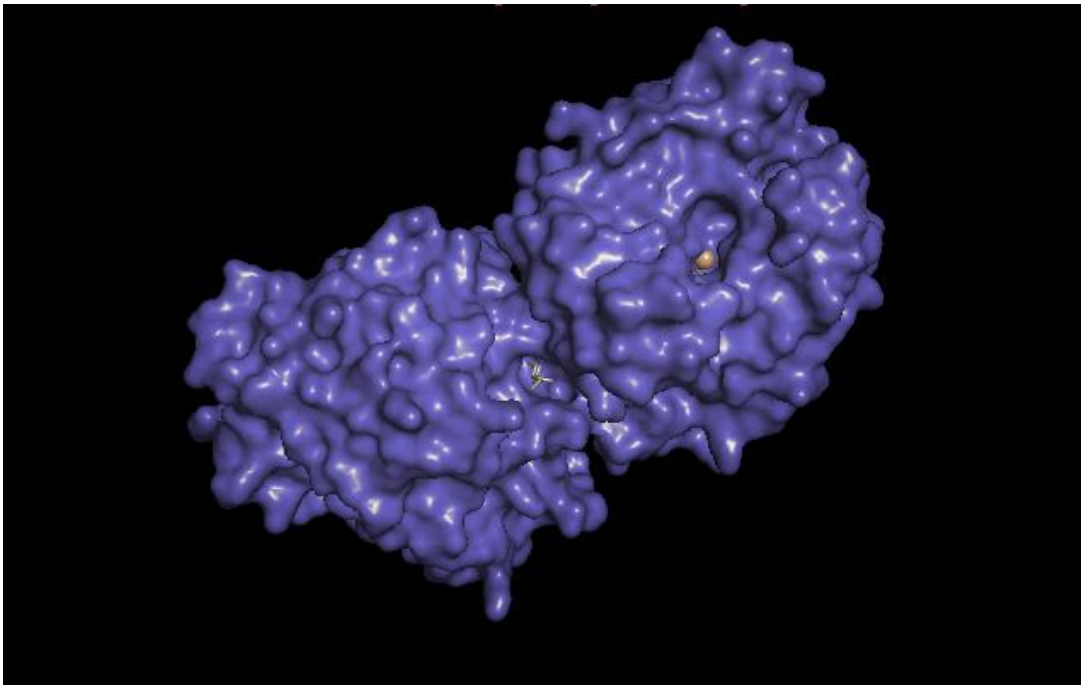


Figure 1.13: Carbonic Anhydrase isozyme XIII Surface and the zinc active center

In fact, carbonic anhydrase is a group of zinc metalloproteins (enzymes) that exists in three genetically unrelated families of isoforms (α, β and γ). Carbonic anhydrases are present in almost all living organisms, from animals, to plants, algae and bacteria. At least 14 genetically distinct -CA isozymes have been identified in human beings. These isozymes have different tissue distributions and intracellular locations. The human variant CA II, located in red blood cells, is the most studied and has the largest catalytic turnover number. It plays a major role in respiration and the blood acid–base balance. In literature, for the catalyzed reaction of CO_2 hydration, a mechanism for CA has been proposed. Above pH 7 the dominant reaction mechanism of carbonic anhydrase with carbon dioxide can be described with (Nathalie J.M.C. Penders-van Elka, 2012) (Byung Hoon Jo, 2013):

- Reaction IV – CO_2–HCO_3^- interconversion

$$CO_2 + EZnOH^- \leftrightarrow EZnOH^-CO_2 \leftrightarrow EZnHCO_3^-$$

$$EZnHCO_3^- + H_2O \leftrightarrow EZnH_2O + HCO_3^-$$

- Reaction V – enzyme regeneration

$$EZnH_2O \leftrightarrow H^+EZnOH^-$$

$$H^+EZnOH^- + B \leftrightarrow EZnOH^- + BH^+$$

The reaction mechanism of catalytic CO_2 hydration as described, results in figure 1.14. This mechanism results in a very complex and long kinetic rate expression.

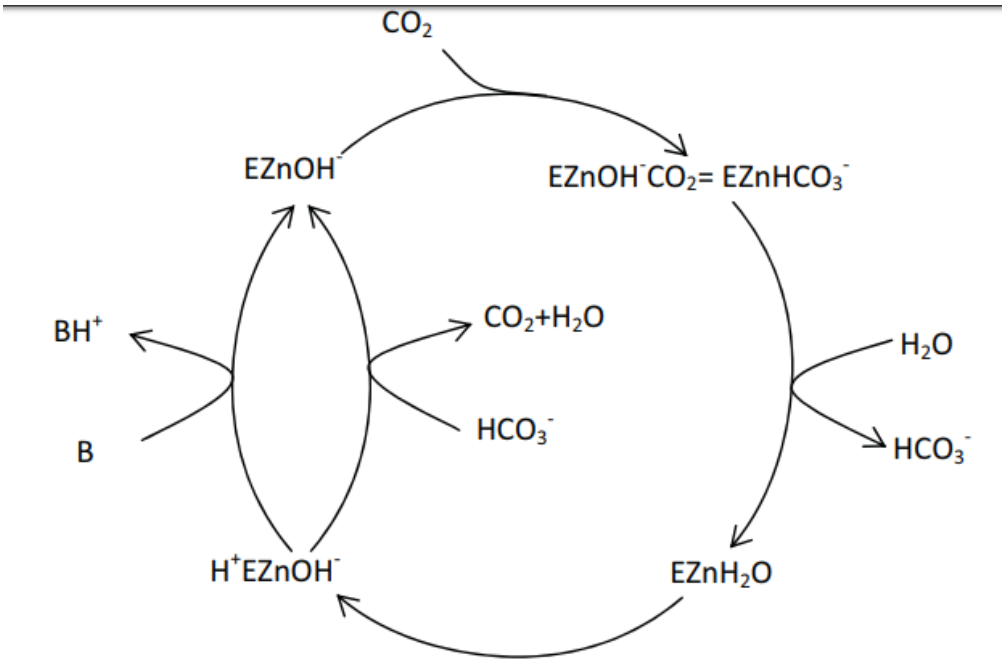


Figure 1.14: Reaction mechanism of catalytic CO_2 hydration by carbonic anhydrase.

Biomimetic utilization of CA as a green route for CO_2 trap method has been proposed and attempted for post combustion CO_2 capture by various entities. However, the use of CA to accelerate carbon capture is limited by the enzyme's sensitivity to the harsh process conditions such as high temperature and basic environment. Using directed evolution, the properties of a β-class CA from Desulfovibrio vulgaris were dramatically enhanced. Iterative rounds of library design, library generation, and high-throughput screening identified highly stable CA variants that tolerate temperatures of up to 107 °C in the presence of 4.2 M alkaline amine solvent at pH >10.0. This increase in thermostability and alkali tolerance translates to a 4,000,000- fold improvement over the natural enzyme. At pilot scale, the evolved catalyst enhanced the rate of CO_2 absorption 25-fold compared with the noncatalyzed reaction. (Oscar Alvizoa, 2014)

In order to enhance stability and reusability techniques like immobilization have also been investigated. Methods and processes using CA would be of primary use at facilities emitting large quantities of CO_2, such as steelworks and power plants. However, in those facilities the use of costly purified enzymes would be unnecessary and economically unfeasible. A convenient solution to the industrial use of CA technology, it might be to employ a microbial whole-cell biocatalyst system harboring efficient CA activity to eliminate the need for cell disruption and enzyme purification, each of which is a potentially high cost

process. That would also provide advantages, such as enzyme stabilization and ease of handling, including simple separation of enzyme. (Byung Hoon Jo, 2013)

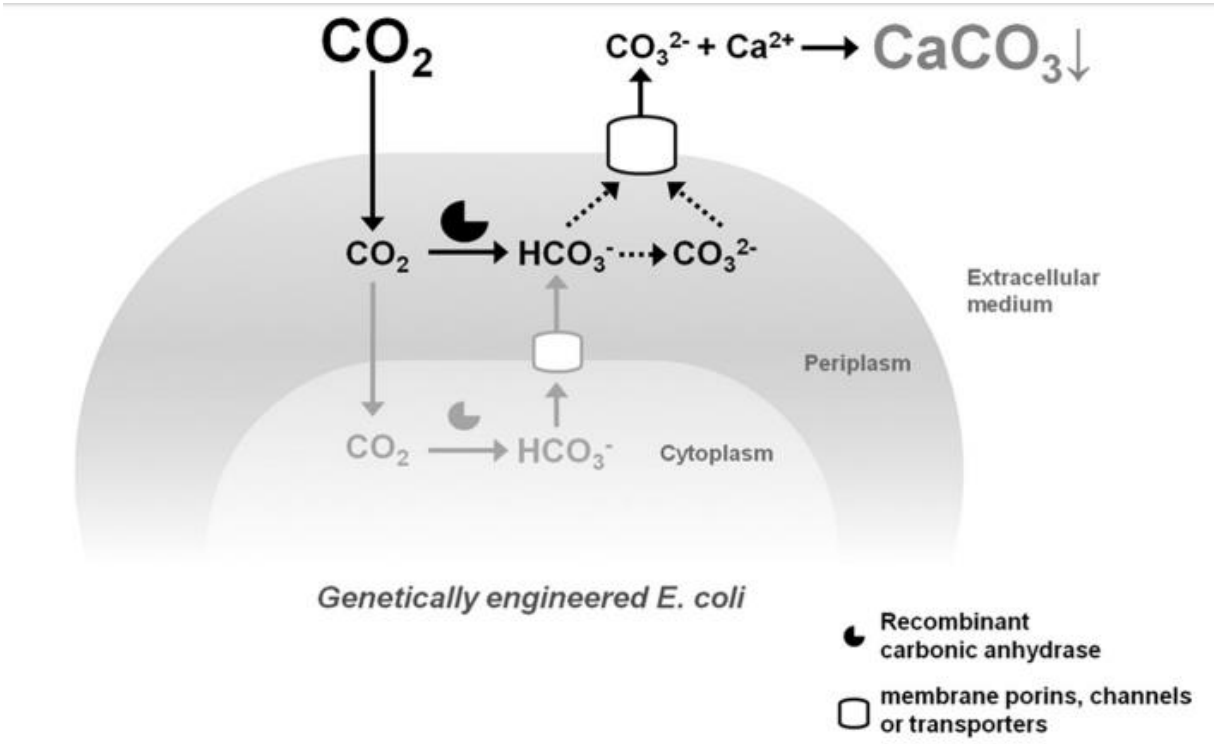


Figure 1.15: Design concept for the engineered periplasmic whole-cell system and its biocatalytic function for CO_2 sequestration. Recombinant CA transforms CO_2 into HCO_3 in the periplasm of E. coli. The anions are transported out of the cell through outer membrane porins or transporters and then react with Ca2 ion to form $CaCO_3$ precipitate. The solid black lines indicate the reaction or transfer of substrates that are experimentally confirmed or are simply deduced. The dotted black lines represent the reactions or transfers that may depend on the extracellular pH. The symbols and lines in gray show the CO_2 pathway via cytoplasmic CA that is hindered by physical (cytoplasmic membrane) or physiological (e.g., pH regulation) barriers.

2.Materials and methods

2.1 Materials

2.1.1. Reagents

The amine solvents and the ionic liquids were provided by Sigma and Aldrich companies. Specifically, N-Methyldiethanolamine 471828 – 250ML by Aldrich company, Pentaethylenehexamine 292753 – 250ML by Aldrich company, Ethanolamine E9508 – 100ML by SIGMA – ALDRICH and L - proline P0380 – 100G by Sigma – Aldrich.

2.1.2. Microorganisms

For the needs of this thesis mutated cells of E. coli were obtained. The mutated gene sequence was prepared according to bibliography references and ordered.

2.1.3. Nutritional medium

For the enzyme expression Luria Broth by Sigma Company (L3522) was used as medium for the carbonic anhydrase (CA08) production and the ZYP-5052 medium. For this medium, the preparation of 4 stock solutions was needed.

Stock solutions

ZY		20xNPS		50x5052	
H_2O		$(NH_4)_2SO_4$	66g/L	γλυκερόλη	250 g/L
Yeast extract	5g/L	KH_2PO_4	136 g/L	γλυκόζη	25 g/L
Tryptone	10g/L	Na_2HPO_4	142 g/L	λακτόζη	100 g/L

1000xTrace elements: 100mL stock solution				
Salt	Concentration	g/mg/ml	MW	Special preparation
$FeCl_3$ x $6H_2O$	50mM	50 ml of 0.1 M stock	270	0.1M stock (V = 100ml) 2.7 g in 1:100 diluted HCl
$CaCl_2$ x2 H_2O	20 mM	0.294 g	147	
$MnCl_2$ x4 H_2O	10 mM	0.198 g	198	
$ZnSO_4$ x$7H_2O$	10 mM	0.288 g	288	
$CoCl_2$ x$6H_2O$	2 mM	47.6 mg	238	
$CuCl_2$ x$2H_2O$	2 mM	34 mg	171	
$NiCl_2$ x$6H_2O$	2 mM	47.5 mg	238	
Na_2MoO_4 x$2H_2O$	2 mM	48.4 mg	242	
H_3BO_3	2mM	400µL of 0.5M stock	61.5	0.5M stock (V= 50ml) = 1.53 g in ca 60mM HCL

For 1 lt of ZYP-5052 medium:

- 928ml ZY
- 1ml MgSO4 (1M)
- 1ml 1000xTrace elements
- 20ml 50x5052
- 50ml 20xNPS
- Ampicillin (final concentration 100µg/ml)

2.1.5 Apparatus and instruments

The instruments and apparatus needed and used for this thesis are referred below:

- Microplate spectrometer reader, Spectromax M2
- Spectrophotometer
- pH-meter
- Incubator
- Stirrer Magnetic
- Hotplate
- Millie Q water device
- Vacuum filtration apparatus
- Centrifuge
- Rotameter
- Flowmeter
- Thermometer
- Manometer
- CO_2, N_2 tank
- Absorption Column
- Heating boiler
- Pump
- Autoclave

Besides the above instruments, various laboratory materials were used, such as volumetric cylinders, beakers, conical flasks, funnels, dishes, Eppendorf tubes, falcon tubes, paster pipettes and glass bottles.

2.2 Methods

2.2.1 Amine solution and ionic liquid preparation

For the amine solutions the preparation occurred on weight of the amine solvent by weight of the solution. The ionic liquid PEHA prolinate was prepared by mixing the amine PEHA and

the amino acid proline, dilute for homogenization and then separate by water using vacuum distillation.

2.2.2. Carbonic Anhydrase Extraction and Stability

Mutated cells of E.coli were obtained and used for the enzyme production. There have been followed two methods for the enzymatic expression that is using IPTG and lactose.

Expression of CA08 using lactose

L. Broth was diluted in Erlenmeyer conic flask in a ratio of 1-5 (1 ml liquid to 5ml air) to final concentration C = 25g /L. The medium was then sterilized and left to cool. 500 μL of mutated E.Coli were inoculated in the flask. Ampicillin was then added, in order to deactivate potential non-mutated cells, to final concentration C=100 μg/ml. The flask was then put in an incubator for 6 – 8 hours at 32^{o}C.

In a second Erlenmeyer conic flask of 1000ml, 200 ml ZYP-5052 medium was used. The starter culture was inoculated in the second flask by dilution analogy 1:100, and the flask was placed back in the incubator for 10 hours.

The solution was then centrifuged in 10000 rpm for 10min, in order to isolate the cells from the medium. The supernatant was removed and the precipitate (cells) was dissolved in bug buster, by analogy to the supernatant 17: 1. Finally, 1-2μL of lysonase was added. The solution was separated in Eppendorf flasks and kept in freezer.

Dry extract using lyophiliosis

A sample of dry enzyme was also used in the experiments in order to investigate whether the state of the enzyme affects its activity and its tolerance.

In this case the precipitate was dissolved in a 100ml buffer of Tris-HCL (0.1M and pH 8.3) and NaCl 0.1 M. The solution is put in the freezer until 4^{o}C and the homogenized at 700bar for 3 cycles. The homogenized solution is let to freeze overnight and then lyophilized.

Activity of the enzymatic extract

The activity of the enzyme extract was calculated by using the titrimetric method. The reaction mixture was prepared in conical flasks under cold conditions by mixing 5μL of liquid enzyme extract or 10mg of dry extract with 1 ml of 0.01M Tris-HCL buffer(pH 8.3) and 2-3

drops of BTB (BromoThymol Blue) indicator with a pH range of 6.0 (yellow) to 7.6 (blue), to which 1ml of CO_2-saturated water was mixed gently and the time for the color change (Tc) from blue to yellow recorded. The non-enzymatic reaction rate was measured by mixing the CO_2-saturated water with the buffer without the enzyme (T_{blank}). The assay was repeated 3 times.

Carbonic anhydrase unit was calculated as (Tb/Tc – 1) and activity was expressed as Units/ml of enzyme, where Tb = Time requested for change in color of blank, Tc = time required for change in color of sample.

Stability test of Carbonic Anhydrase

In order to assure that the expressed carbonic anhydrase has the desired properties, that is resistibility to high temperatures and extreme pH values, a series of stability tests were required. The activity of the carbonic anhydrase was tested in different temperatures and in basic environment.

For stability test in high temperatures and basic environments a stability method based on the pH change in the solution, was developed. This method is able to measure the activity of the enzyme extract through the rate of a specific reaction. The reaction is the thermal decomposition of potassium hydro carbonate, as shown below:

$$KHCO_3 \rightarrow K_2CO_3 + CO_2\uparrow + H_2O$$

Carbonic anhydrase is used as a catalyst for the reaction making it possible to occur in room temperature. In order to measure the rate of the reaction, phenolphthalein is needed as an indicator. The spectrometer was used to measure the absorbance of the solution at 550nm (A_{550}) and thus the rate of the reaction. A solution of non-mutated cells of E. coli was used as control to measure the rate of the reaction without the enzyme. The activity was calculated as the difference of the enzymatic rate and the non-enzymatic rate.

The enzyme extract was inoculated in Eppendorf flasks and incubated at the specified temperatures for 24 hours. A 300 mM $KHCO_3$ solution with adjusted pH 8.0 was used as buffer. A phenolphthalein solution (1% by mass) was used as indicator. In the microplate well 192.5 μl of buffer and 2.5 μl of phenolphthalein were added. The microplate reader was

set on kinetic mode, 550nm. 2.5 µl of sample (enzyme extract/ control) were added last in the well and the microplate was placed in the reader for 20min.

The next step was to test the enzyme stability in both basic environment and high temperatures. That is an important step because the amine solvent – enzyme solution will be used in higher temperatures than the room temperature that is temperatures from 40 °C to 80 °C.

2.2.3 CO_2 Absorption in amine solvents

Absorption tests in tiny clave equipment

The tested amines solvents are widely used for post-combustion CO_2 capture applications, especially in power plants. It is proved that amine solvents have a high CO_2 load capacity. Absorption tests for the total CO_2 load capacity with and without the carbonic anhydrase performed in order to check if the presence of the enzyme in the solvent changes the total load capacity. The method used is based on the CO_2 pressure drop from a start point of 300 mbar in a closed chamber filled by solvent sample. During the run, the chamber was filled with 99.9% CO_2 gas. The solvent absorbs CO_2 causing pressure drop. A manometer saves the pressure data in a computer for a desired period.

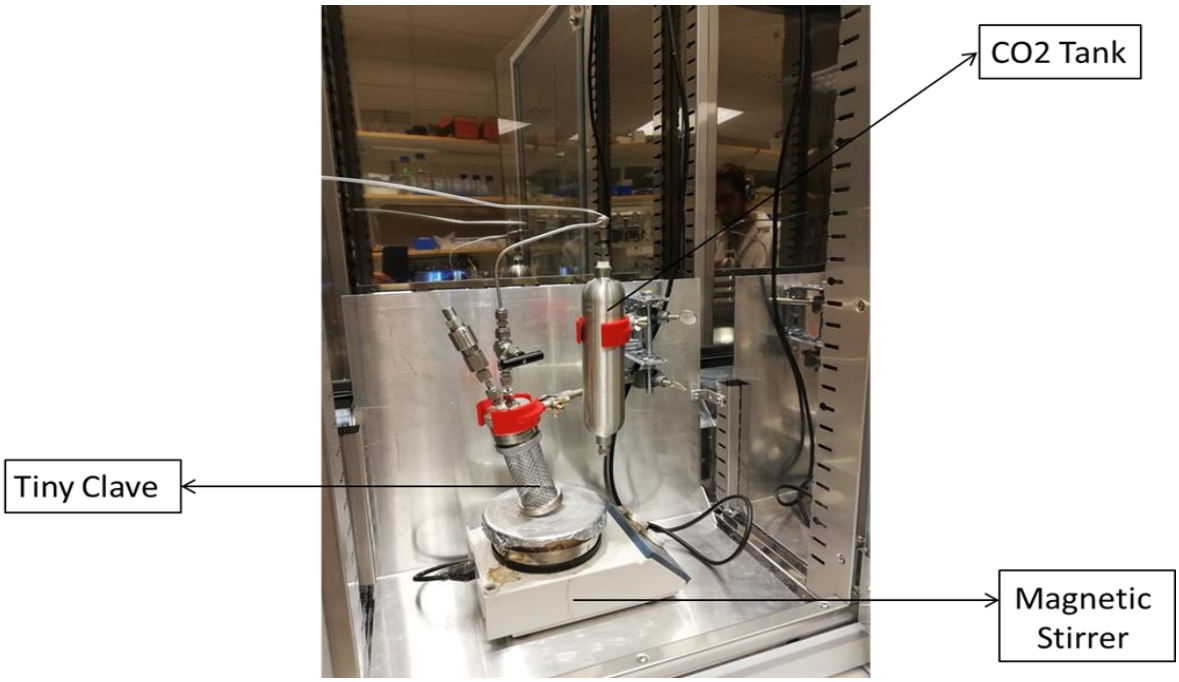


Figure 2.1: Tiny clave incubator equipment

Samples of 25% w/w amine solutions of the MDEA, PEHA, MEA with addition of enzyme solution 2% w/w, were prepared and weighed.

The equipment was prepared by flushing the lines with CO_2 and purging the CO_2 reservoir. The sample was then placed in the equipment. Before opening the valve to the sample chamber, purging occurred once more. The valve opens and the manometer starts gathering data of the pressure drop. After the run, the sample was weighed again. The difference in grams before and after the run was the amount of absorbed CO_2.

Absorption tests performed for each amine solvent in samples with and without enzyme solution added for 24h, in order to measure the saturation point. More tests performed for 30min in order to measure the absorption rates again with and without enzyme. A solution of non-mutated cells was used as control. Each sample was tested in triplicates and after statistical study the average absorbed grams were calculated.

Scaled up equipment for absorption and stripping experiments

The results from the absorption experiments acted positive to continue on scaling up the equipment. The new equipment was designed to provide information about the absorption rates and thus the quantity of CO_2 absorbed for higher volumes of solvent solution. It was also designed to provide information for the stripping rates of CO_2 from the solution.

The equipment was designed to operate in atmospheric pressure and temperatures up to 100 °C. It consisted of the main chamber, a condenser, a rotameter, a flowmeter in the outlet and a CO_2 analyzer. For the stirring a magnetic stirrer was used. The temperature was checked throughout the experiment by a thermometer and maintained by a heat bath.

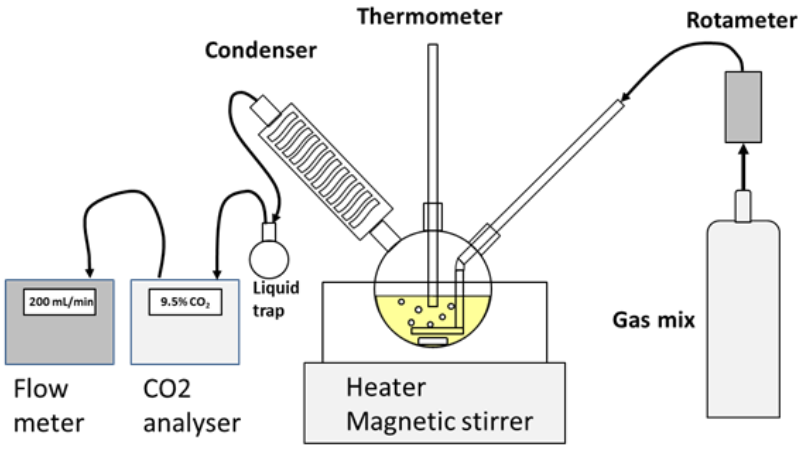


Figure 2.2: Scaled-up equipment model presentation

Before starting the experiment, a CO_2–N_2 gas mixture, containing 20 vol % CO_2 with a flowrate of 400mL min^{-1}, was circulated through a by–pass valve to calibrate the analyzer and for purging the system. For the absorption part, the bath was heated up to 40 ± 0.5 ^oC and the stirrer was set at 300rpm. The gas mixture was passed through the solvent solution with the flow set at 500mL min^{-1}. The solution consisted of 100 ml amine solvent with and without enzyme (control). The liquid enzyme quantity added was to final concentration of 1% in weigh. Dry enzyme was also used in different concentrations.

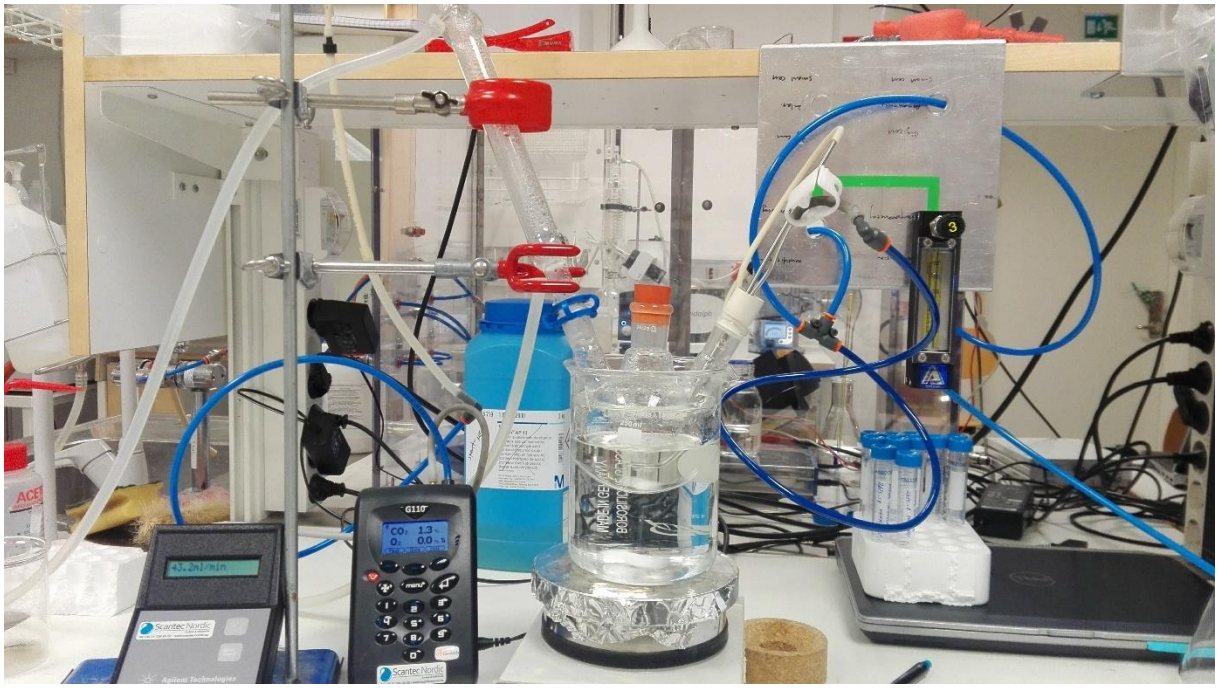


Figure 2.3: The prepared scaled up equipment that was used for the experiments

For the stripping part to start, the N_2 gas flow was switched on and used to purge the system until the CO_2 and O_2 outlet are close to 1%. Meanwhile the bath was heated to 80°C. At 80°C, the N_2 gas was connected to the main line, the stirrer was switched on again at 300 rpm and the flow was set at 200 mL min^{-1}.

It is important to mention that, sometimes, during the experiment the presence of the bug buster in combination with the bubbling led to foaming. In order to prevent any liquid leak into the analyzer a liquid trap was used after the condenser.

The rate of CO_2 absorption/stripping at a given time was computed from (U. E. Aronu, 2009):

$$Q_{CO_2}\left[\frac{mol\ CO_2\ in\ solution}{s}\right] = \left(n_{CO_2}^{in}\left[\frac{mol\ CO_2}{s}\right] - \frac{x_{CO_2}^{out}[mole\ fraction]n_{N_2}\left[\frac{mol\ N_2}{s}\right]}{(1-x_{CO_2}^{out})[mole\ fraction]}\right)$$

The moles of CO_2 were calculated based on ideal gas law for atmospheric pressure and temperature 40°C and 80°C for absorption and stripping respectively. Two plots were designed in order to compare the difference between the control and the enzyme solution. The first one presents the CO_2 concentration increase over time. The second one presents the change of the absorption rate over the concentration increase. Because of the high pH intensity of the MEA, experiments in lower concentration were considered necessary in order to remark any difference cause by the enzyme extract presence.

Absorption Tests on scaled-up automated pilot plant

The next step was to scale up to an industrial rig as shown in picture. A filling column was used for the absorption. The was provided by HFT.

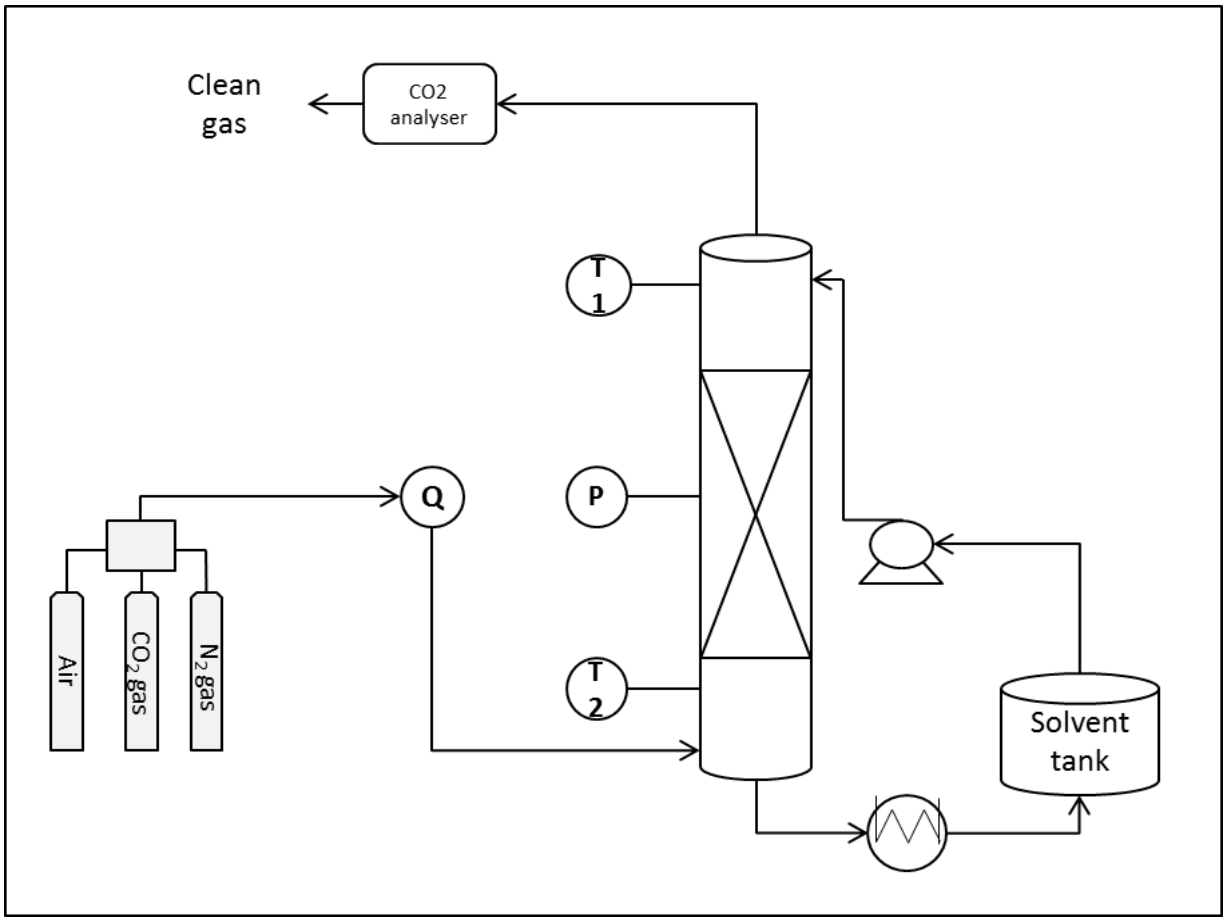


Figure 2.4: Automated absorption/desorption pilot plant model

The amine solvent was introduced by the upwards of the column. For the absorption part a gas mix of 14.3 % CO_2 was introduced by the downwards of the column. For the stripping part Nitrogen (N_2) was used as a drift gas. The solvent flow was set to 24.0 L/min, the gas mix flow was set to 4.2 L/min and the N_2 flow was set to 2.3 L/min. The temperature in the system was measured by two thermometers in the inlet and the outlet of the amine solvent. In the gas mix outlet, the percentage of the CO_2 was measured by the CO_2 analyzer. A water boiler was used for heating. The absorption temperature was set at 40^oC and for the stripping at 80^oC. During the process samples were taken from a valve in the middle of the column and the pH value was measured. After the pH measurement each sample was analyzed by the precipitation–titration method ($BaCl_2$ method) and the concentration of the CO_2 was measured.

Figure 2.5: Automated absorption/desorption pilot plant

The precipitation–titration method ($BaCl_2$ method)

The liquid sample was added to a 250 cm^3 Erlenmeyer flask containing 50 cm^3 sodium hydroxide (NaOH, 0.1 mol L^{-1}) and 25 cm^3 barium chloride ($BaCl_2$, 0.5 mol L^{-1}) solutions. The amount of the liquid sample added depended on the total CO_2 content of the sample. The Erlenmeyer was heated to enhance the barium carbonate ($BaCO_3$) formation and then cooled to ambient temperature. The mixture was filtered with a 0.45 µm Millipore paper and washed with deionized water. The filter covered by $BaCO_3$ was transferred to a 250 cm^3 beaker. Deionized water, 50 cm^3, was added into the beaker and enough hydrogen chloride (HCl, 0.1 mol L^{-1}) was also added to dissolve the $BaCO_3$ cake. The amount of HCl not used to dissolve $BaCO_3$ was then titrated by NaOH, 0.1 mol L^{-1}, with end point pH value of 5.2 (indicator).

3.Results and Discussion

3.1 Carbonic anhydrase activity and stability

Carbonic anhydrase unit was calculated as (R.R. Warrior, 2014):

$$Units = (T_{blank}/T_{control} - 1)$$

The activity was expressed as Units/µl of enzyme, where T_{blank} = Time requested for change in color of blank, $T_{control}$ = time required for change in color of sample.

Table 3-1: Sample time (Tc) and blank time (Tb) of color changing in liquid enzyme's activity calculation method

Carbonic Anhydrase Activity (units/µl)			
	Tc (sec)	Tb (sec)	Units
X_1	1.67	41.42	
X_2	1.83	41.13	22.57
X_3	1.79	42.11	Activity (units/µl enzyme)
SD	0.083	0.503	
Average	1.76	41.55	4.5

Table 3-2: Sample time (Tc) and blank time (Tb) of color changing in 6mg of dry enzyme's activity calculation method

Carbonic Anhydrase Activity (units/mg)			
	Tc (sec)	Tb (sec)	Units
X_1	1.54	18.74	
X_2	1.17	19.06	12.44
X_3	1.52	19.08	Activity (units/mg enzyme)
SD	0.208	0.191	
Average	1.41	18.96	2.07

The results, based on the method for the enzyme's stability, are shown in table 3-3.

Table 3-3: Gradient of absorption curve through time of enzyme and control samples in room temperature (RT), 60°C and 90°C for 24h.

	RT			60°C			90°C		
time	*10 min*	*3h*	*24h*	*10 min*	*3h*	*24h*	*10min*	*3h*	*24h*
enzyme	0,0435	0,0532	0,0416	0,0411	0,0369	0,0347	0,0244	0,0269	0,0121
control	0,0084	0,017	0,0076	0,0107	0,0048	0,0064	0,0128	0,0085	0,0101
diff	0,0351	0,0362	0,034	0,0304	0,0321	0,0283	0,0116	0,0184	0,002

The activity of the carbonic anhydrase through time is shown in figure 3.1.

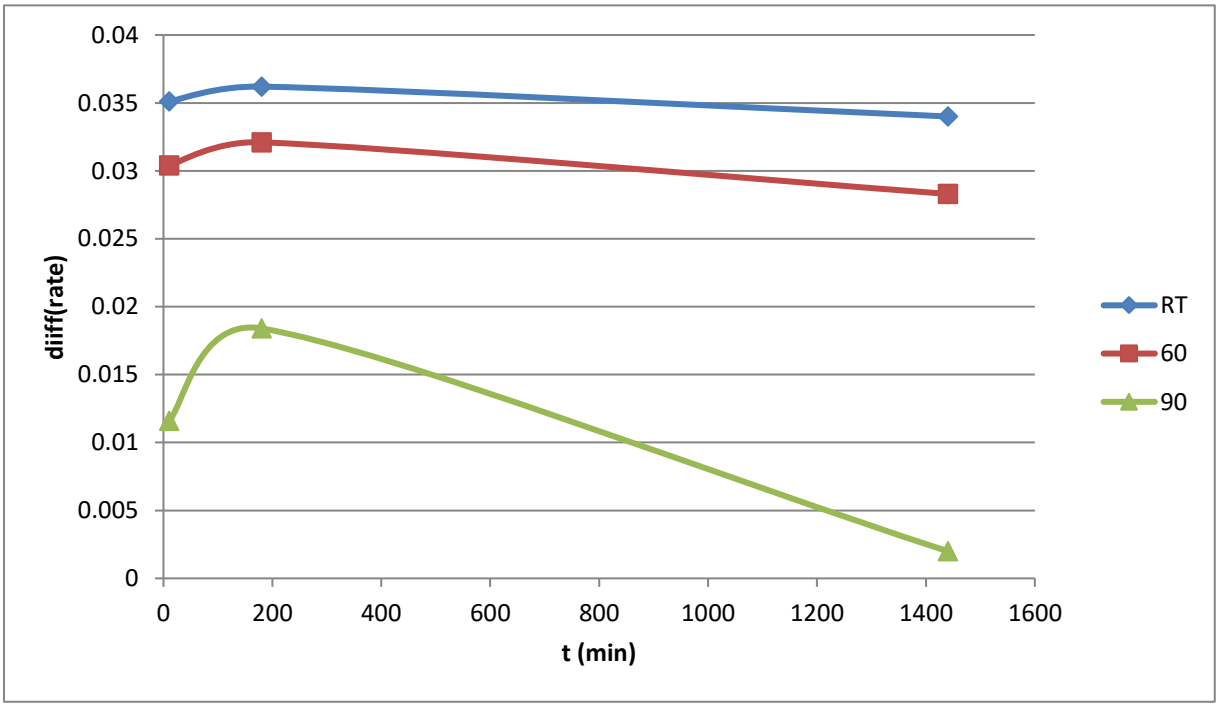


Figure 3.1: Activity loss of enzyme samples during thermal stress tests in room temperature (RT), 60°C and 90°C

It is obvious that for both the room temperature and the 60°C the activity of the enzyme is quite stable. The activity remains high for a period in 90°C.

The stability in basic environments is shown as the activity loss of the carbonic anhydrase in 25% w/w aqueous solutions of amines (MDEA, PEHA, MEA).

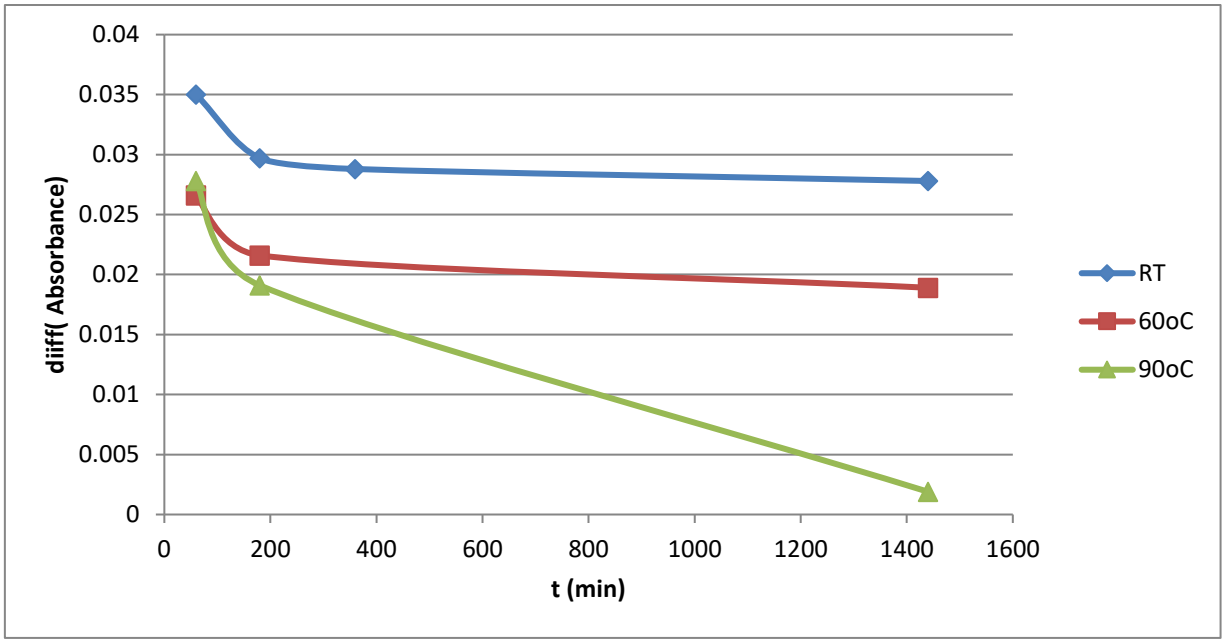


Figure 3.2: Stability test of enzyme extract activity in basic MDEA 25%w/w solution during thermal stress tests in room temperature (RT), 60°C and 90°C

The enzyme remains active both in room temperature and 60°C in MDEA solution and it follows the same tense in both cases. It has also a good activity at 90°C for a satisfying time period. The same is observed for 60°C in PEHA solution.

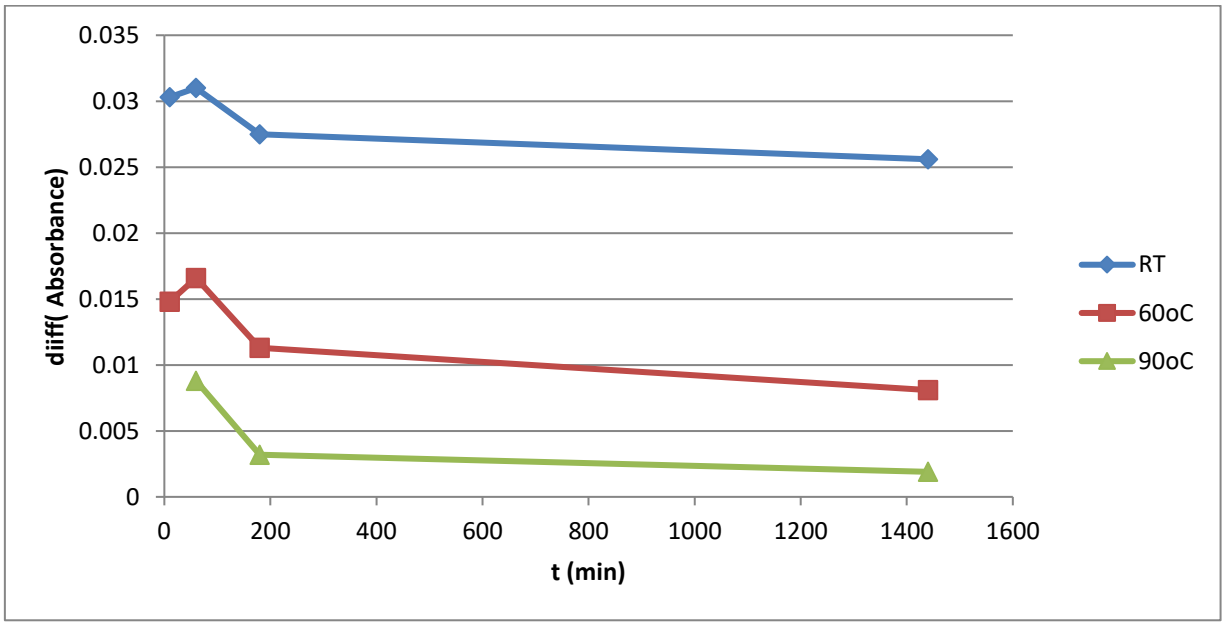


Figure 3.3: Stability test of enzyme extract activity in basic PEHA 25% solution during thermal stress tests in room temperature (RT), 60°C and 90°C

However, the activity loss comparing to the one in room temperature is significant. In the same amine solution and in 90°C the environments conditions are too harsh for the enzyme.

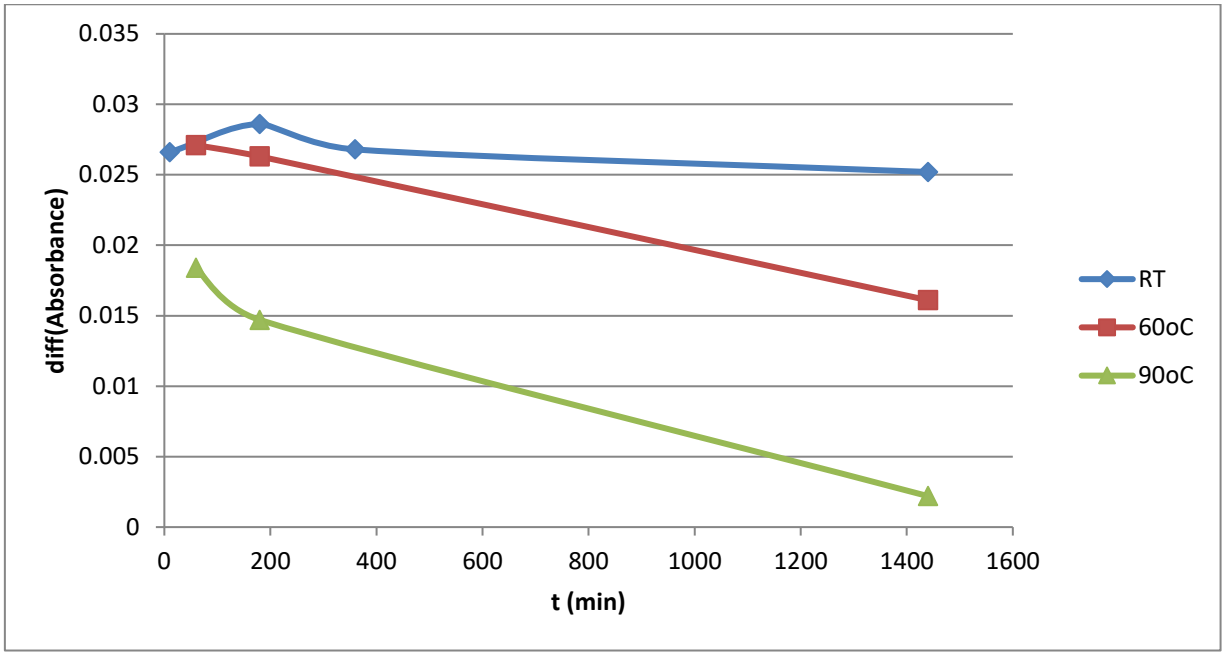


Figure 3.4: Stability test of enzyme extract activity in basic MEA 25% solution during thermal stress tests in room temperature (RT), 60°C and 90°C

The enzyme stays active both in room temperature and in 60°C in MEA solution. However, the loss rate is high in 60°C. The enzyme was deactivated after 20h in 90°C.

The thermostability tests in basic environment show that, although, there is activity loss compared to the room temperature, the enzyme remains stable for all three amine solutions in 60°C. It is also observed that the enzyme was deactivated for all amine samples in 90°C.

The activity of the carbonic anhydrase in basic environment of different amine solvents in room temperature is shown in figure 3.5.

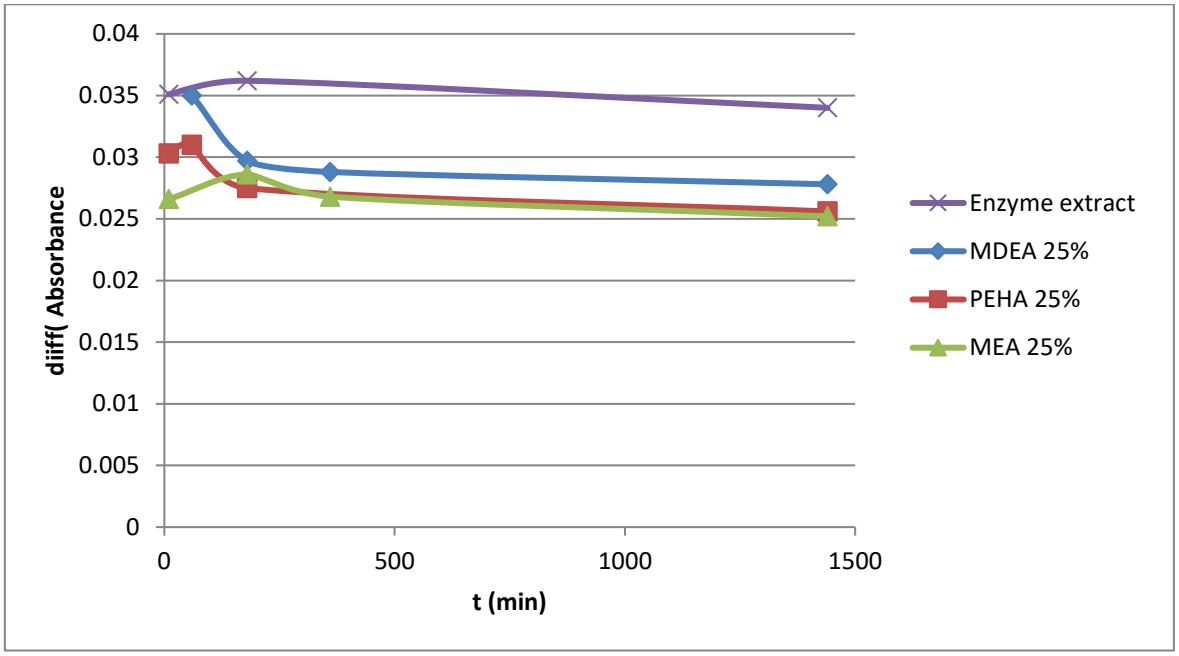


Figure 3.5: Total plot of stability test of enzyme extract activity in different amine solutions during thermal stress tests in room temperature (RT), 60°C and 90°C

There is a loss in the activity of the enzyme however it is still active when in a basic environment. It has been remarked that the enzyme needs a period of 180min in order to stabilize.

3.2. CO_2 Absorption in amine solvents

3.2.1. Tiny clave equipment

Saturation Points of the amine solutions MDEA 25%, PEHA 25%, MEA 25% and the ionic liquid PEHA prolinate 25% with and without enzyme

The first step was to measure the CO_2 capacity for the different amine solutions. The data, from the manometer, for the amines saturation points and absorption rates are shown in figure 3.6.

Each sample was tested in triplicates and after statistical study the average pressure was calculated for aqueous solutions of MDEA (25% w/w), PEHA (25% w/w), MEA (25% w/w) and PEHA prolinate (25% w/w).

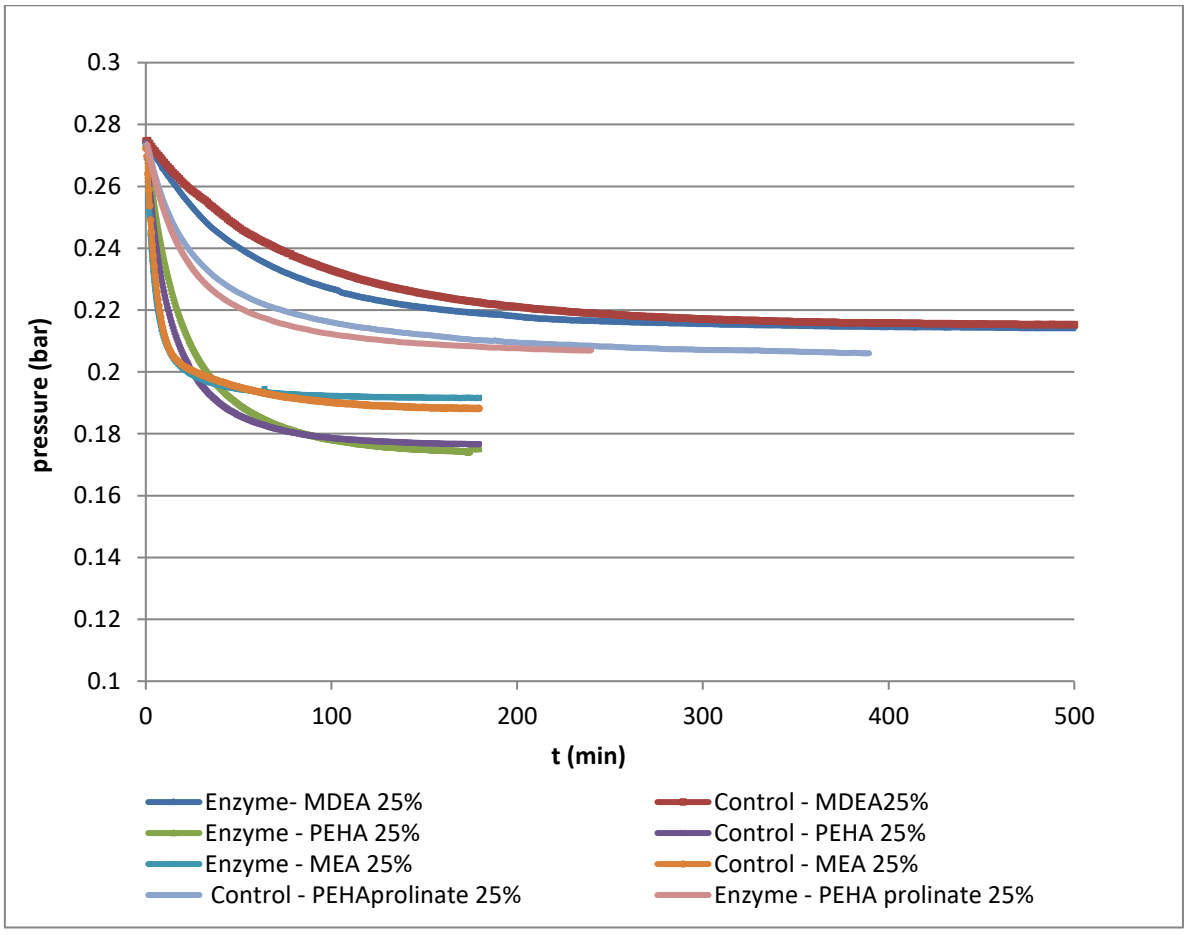


Figure 3.6: Pressure drop in tiny clave because of CO_2 absorption in amine solvent samples with enzyme extract and control extract samples until saturation point has been reached.

The gravimetrical results calculated by the difference in grams before and after the absorption test are shown in table 3-4. Considering the difference on the fourth digit as a statistical error, there is no difference in the CO_2 load capacity of the solution with and without the enzyme addition for all three solvents.

Table 3-4: Gravimetrical results of absorbed CO_2 into amine solvents for enzyme and control samples

		MDEA 25%	**PEHA 25%**	**MEA 25%**	**PEHA prolinate 25%**
Enzyme 2% w/w	Sample 1	79.8 $mgCO_2/g$ sample	125.3 $mgCO_2/g$ sample	110.5 $mgCO_2/g$ sample	82.3 $mgCO_2/g$ sample
	Sample 2	82.1 $mgCO_2/g$ sample	120.2 $mgCO_2/g$ sample	106.3 $mgCO_2/g$ sample	82.6 $mgCO_2/g$ sample
	Sample 3	80.2 $mgCO_2/g$ sample	120.4 $mgCO_2/g$ sample	117.0 $mgCO_2/g$ sample	83.3 $mgCO_2/g$ sample
	Mean	**80.7 $mgCO_2/g$ sample**	**122.0 $mgCO_2/g$ sample**	**109.5 $mgCO_2/g$ sample**	**82.7 $mgCO_2/g$ sample**
	Standard deviation	0.0012	0.0029	0.0029	0.0005
	CV (=S.D./MEAN)	0.0155	0.0235	0.0263	0.0066
Control	Sample 1	83.9 $mgCO_2/g$ sample	121.7 $mgCO_2/g$ sample	110.5 $mgCO_2/g$ sample	81.2 $mgCO_2/g$ sample
	Sample 2	83.5 $mgCO_2/g$ sample	121.4 $mgCO_2/g$ sample	108.1 $mgCO_2/g$ sample	83.5 $mgCO_2/g$ sample
	Sample 3	74.0 $mgCO_2/g$ sample	123.2 $mgCO_2/g$ sample	109.6 $mgCO_2/g$ sample	83.7 $mgCO_2/g$ sample

	Mean	**80.5 mgCO_2/g sample**	**122.1 mgCO_2/g sample**	**109.4 mgCO_2/g sample**	**82.8 mgCO_2/g sample**
	Standard deviation	0.0056	0.0010	0.0012	0.0014
	CV (=S.D./MEAN)	0.0696	0.0078	0.0111	0.0169

It is obvious that for all the amine solvent solutions the presence of the enzyme does not lead to a different saturation point. That can be confirmed both from the CO_2 pressure drop and the gravimetrical measurements for the saturation points tests.

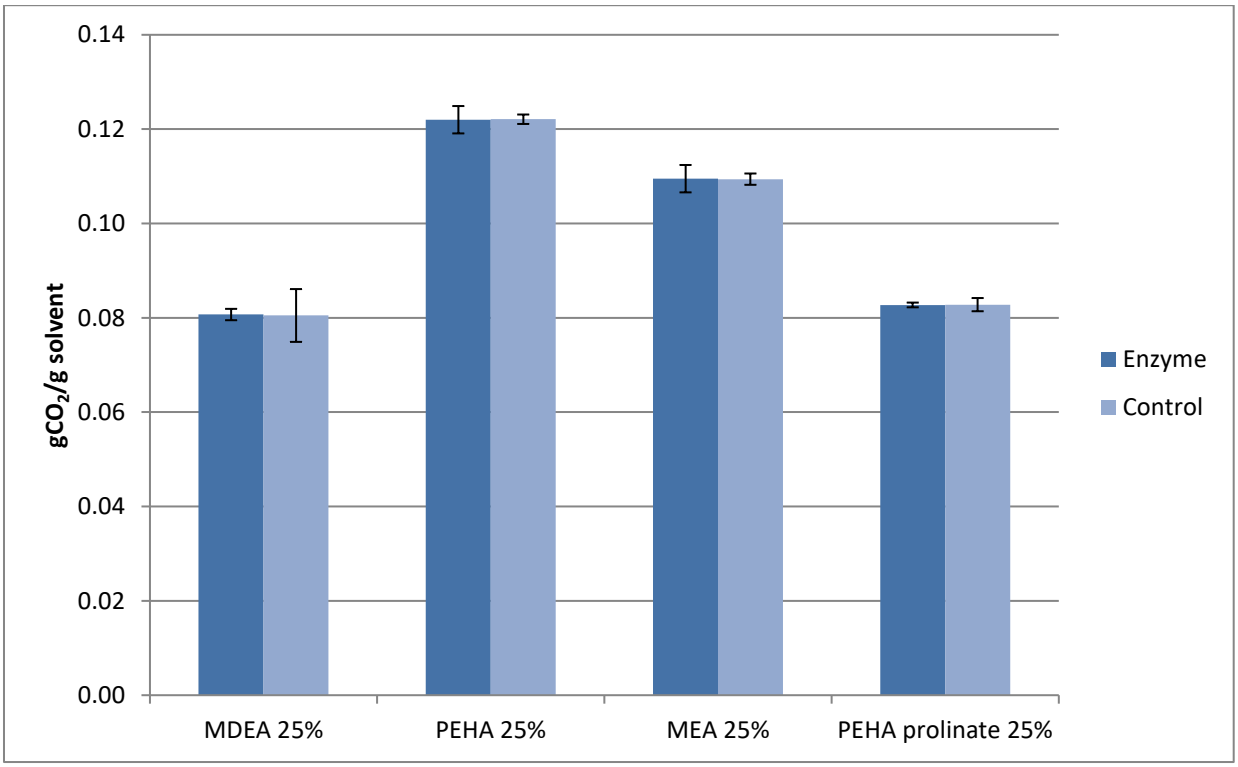


Figure 3.7: Significant error on saturation points of the amine solutions with and without enzyme (control)

Absorption rates of the aqueous solutions of MDEA (25% w/w), PEHA (25% w/w), MEA (25% w/w) and the ionic liquid solution PEHA prolinate (25% w/w) with and without enzyme

In order to calculate the absorption rate new absorption tests were performed for each amine solvent for 30min. A solution of non-mutated cells was used as control.

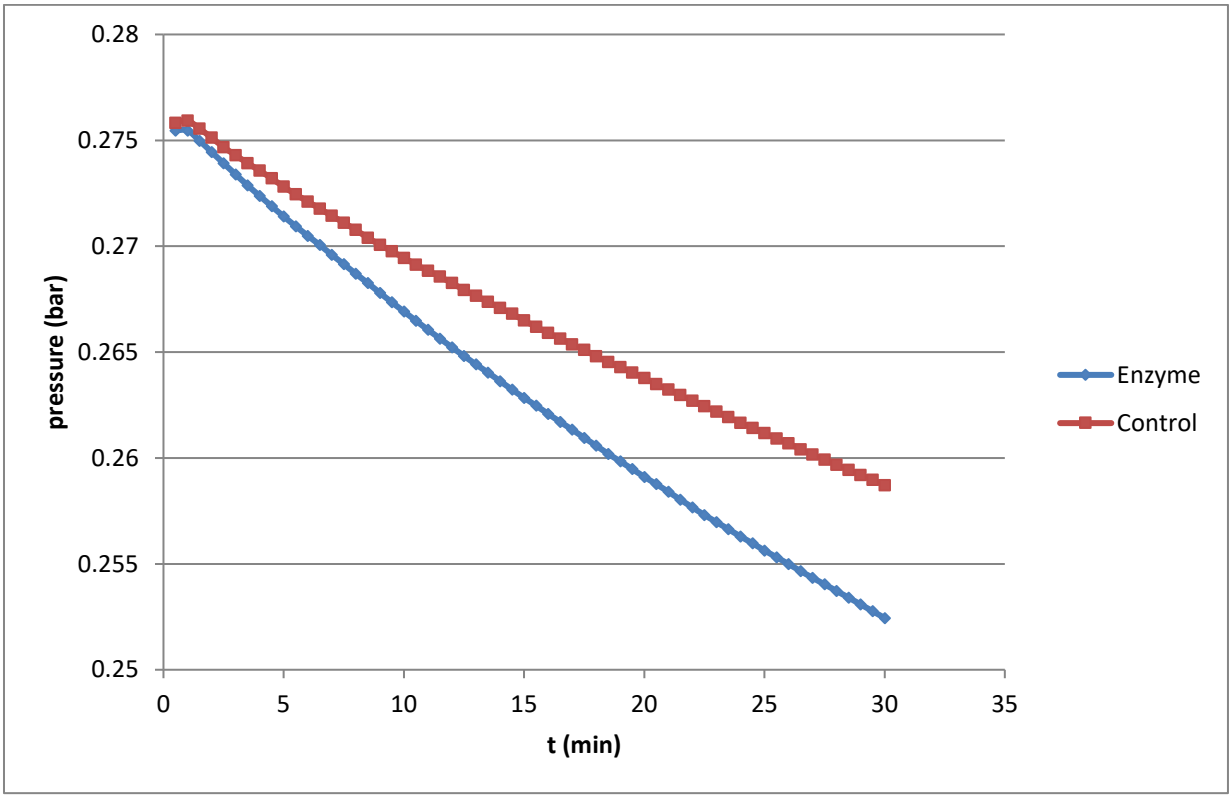


Figure 3.8: Rate of pressure drop in MDEA 25% solution with and without enzyme (control)

For all the three solvents the difference between the pressure drop of the enzyme solution and the control solution is distinguishable.

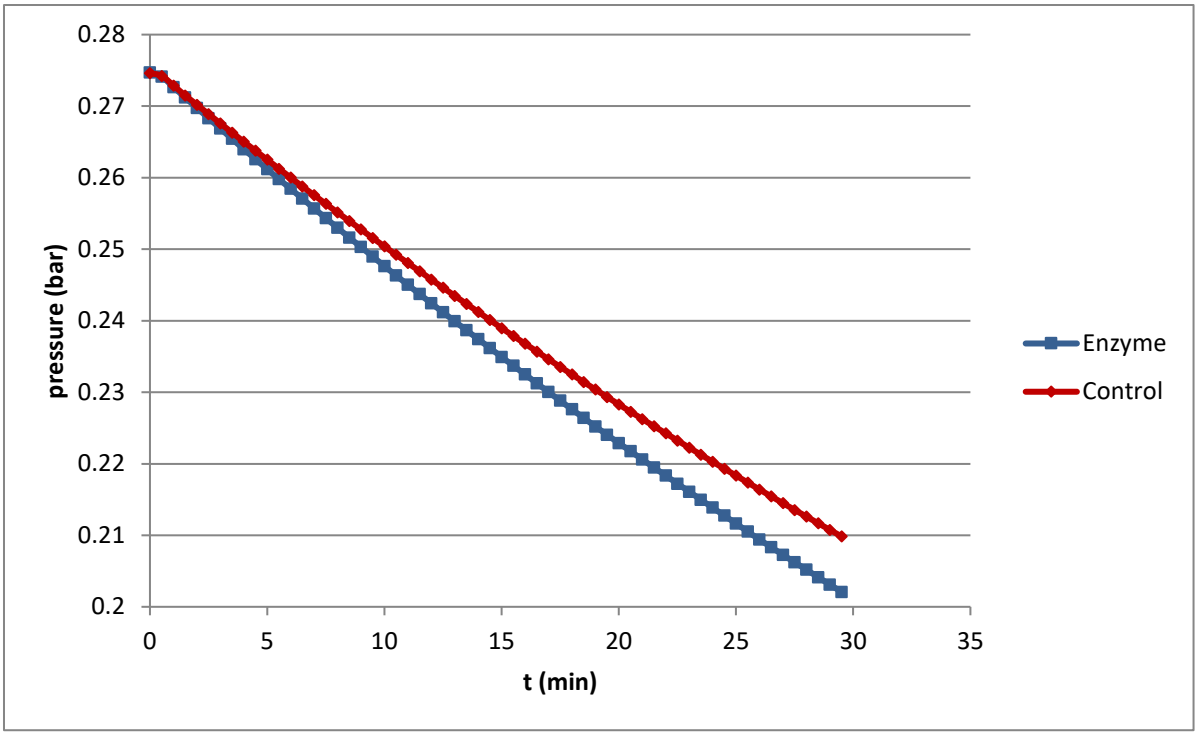


Figure 3.9: Rate of pressure drop in PEHA 25% w/w solution with and without enzyme (control)

It is observed that the effect of the enzyme is higher for the MDEA. For both the PEHA and the MEA, the effect was weaker concerning the pressure drop but intense enough concerning the gravimetrical results.

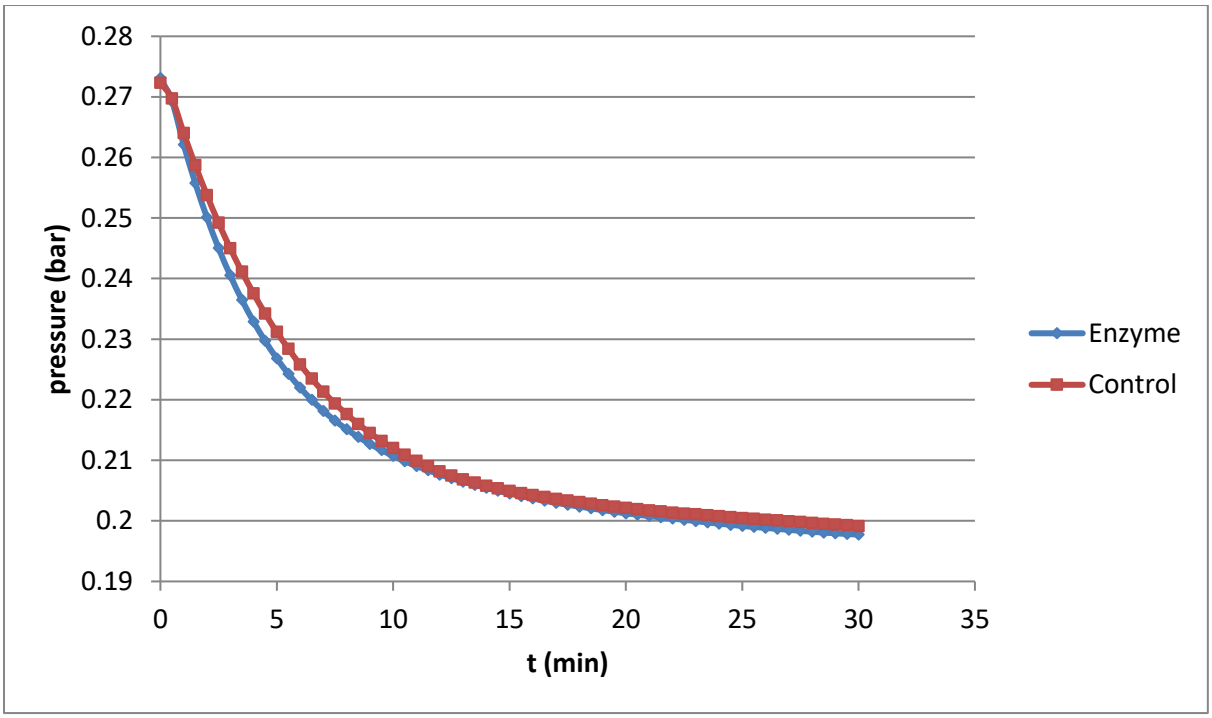


Figure 3.10: Rate of pressure drop in MEA 25% solution with and without enzyme (control)

However, the pressure drop on the PEHA prolinate solution shows no difference between the enzyme sample and the control.

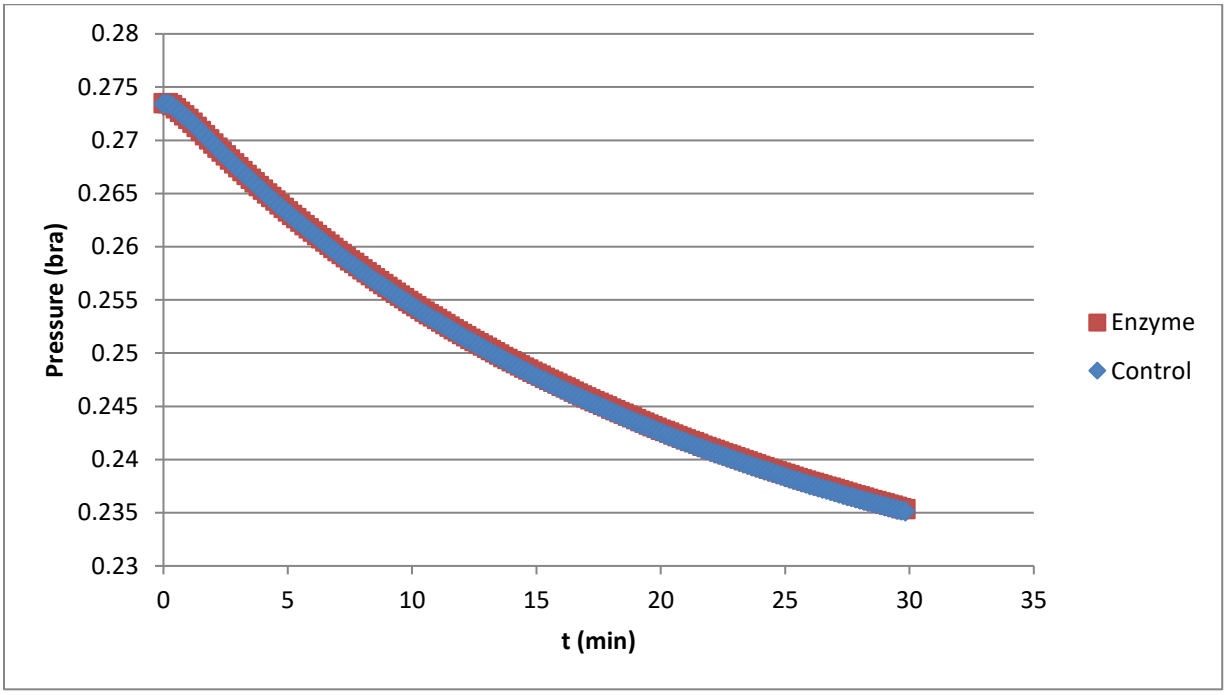


Figure 3.11: Rate of pressure drop in PEHA prolinate 25% w/w solution with and without enzyme (control)

In figure 3.12 the total pressure drop for all three solvents is presented. It is obvious that higher absorption rate occurs for the MEA. However, for the MDEA and the PEHA, the enzyme effect is higher than the one in the MEA. Especially for the PEHA the enzyme increases the absorption rate leading to the same pressure drop as the MEA during the same time of period. Specifically, the presence of the enzyme increases the absorption rate by 40% for the MDEA (25% w/w), 9.32% for the PEHA (25% w/w), 8.01% for the MEA (25% w/w) and 4.88% for the ionic liquid PEHA prolinate (25% w/w).

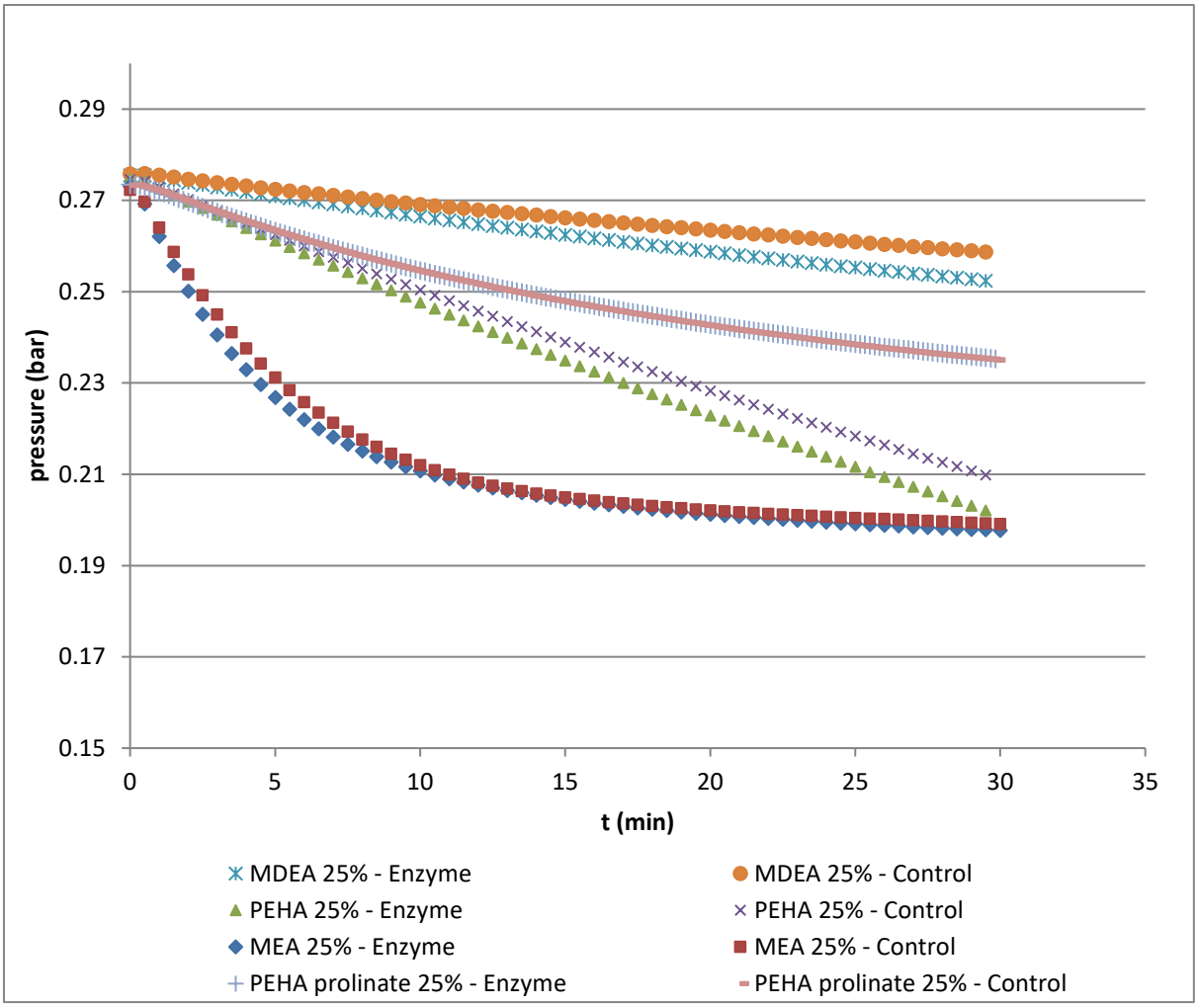


Figure 3.12: Total pressure drop because of CO_2 absorption in amine solvents

The gravimetrical results for the absorption rate are shown in table 3-5. For all three MDEA, PEHA, MEA solvents the gravimetrical results follow the pressure drop results. In contrast, the gravimetrical results for the absorption rate of the PEHA prolinate show a different rate in the enzyme solution and the control solution when the pressure drop results show no difference between the rates. That gravimetrical difference is shown in graph.

Table 3-5: Gravimetrical results from absorption experiment measured by pressure drop in different amines solvents

		MDEA 25%	PEHA 25%	MEA 25%	PEHA prolinate 25%
Enzyme 2% w/w	Sample 1	0.96 $mgCO_2/g$ sample/min	3.03 $mgCO_2/g$ sample/min	3.36 $mgCO_2/g$ sample/min	1.72 gCO_2/g sample/min
	Sample 2	1.06 $mgCO_2/g$ sample/min	3.06 $mgCO_2/g$ sample/min	3.45 $mgCO_2/g$ sample/min	1.70 $mgCO_2/g$ sample/min
	Sample 3	1.13 $mgCO_2/g$ sample/min	3.06 $mgCO_2/g$ sample/min	3.32 $mgCO_2/g$ sample/min	1.75 $mgCO_2/g$ sample/min
	Mean	**1.05 $mgCO_2/g$ sample/min**	**3.05 $mgCO_2/g$ sample/min**	**3.37 $mgCO_2/g$ sample/min**	**1.72 $mgCO_2/g$ sample/min**
	Standard deviation	0.00009	0.00002	0.00006	0.00003
	CV (=S.D./MEAN)	0.08322	0.00672	0.01972	0.01505
control	Sample 1	0.73 $mgCO_2/g$ sample/min	2.74 $mgCO_2/g$ sample/min	0.00313 $mgCO_2/g$ sample/min	0.00167 $mgCO_2/g$ sample/min
	Sample 2	0.78 $mgCO_2/g$ sample/min	2.83 $mgCO_2/g$ sample/min	3.15 $mgCO_2/g$ sample/min	1.60 $mgCO_2/g$ sample/min
	Sample 3	0.73 $mgCO_2/g$ sample/min	2.80 $mgCO_2/g$ sample/min	3.08 $mgCO_2/g$ sample/min	1.64 $mgCO_2/g$ sample/min

	Mean	**0.75 mgCO_2/g sample/min**	**2.79 mgCO_2/g sample/min**	**3.12 mgCO_2/g sample/min**	**1.64 mgCO_2/g sample/min**
	Standard deviation	0.00003	0.00004	0.00003	0.00003
	CV (=S.D./MEAN)	0.04262	0.01642	0.01156	0.01959

In order to assure that there is a significant difference between the enzyme and the control solution of PEHA-prolinate (25% w/w) absorption rate, a statistical analysis occurred. Specifically, single factor anova was used in excel.

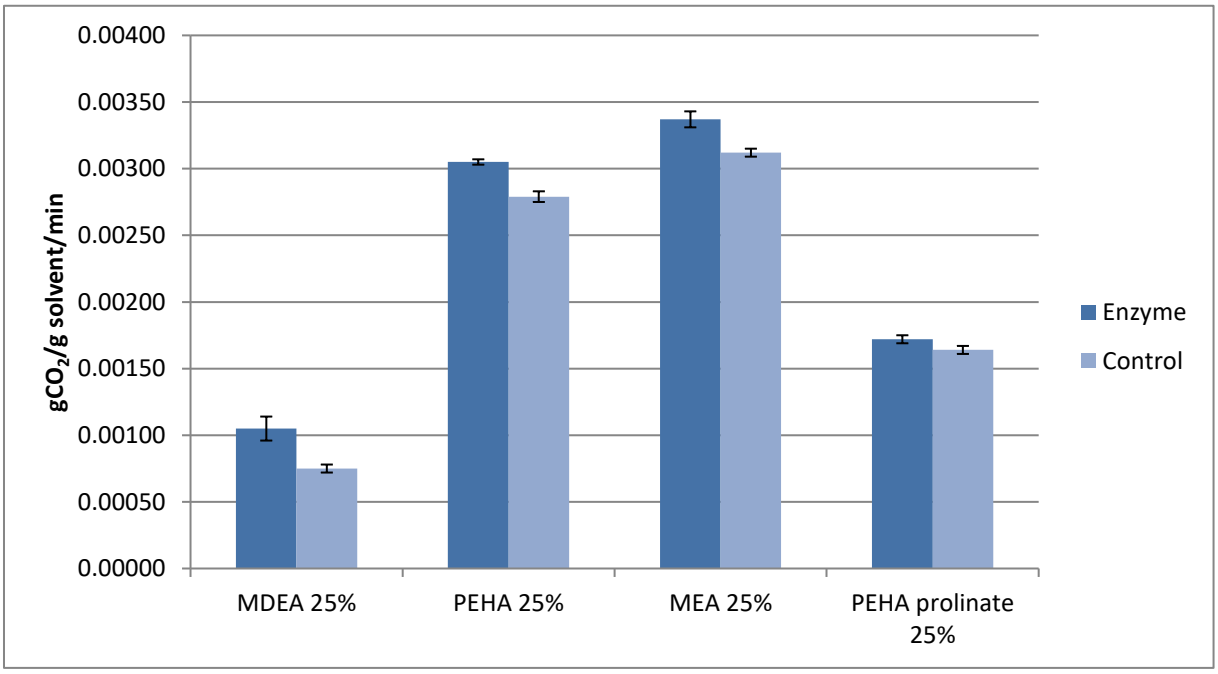


Figure 3.13: Significant error on absorption tests in different amine solutions with and without enzyme (control) for 30min

The significant value or P-value is below 0.05 and, therefore, there is a statistically significant difference between the two means. That means that the enzyme has a light influence on the absorption rate in PEHA-prolinate solution as well.

Table 3-6: Statistical analysis on the absorption test in PEHA prolinate solution in order to check if there is a significant difference between the enzyme and the control sample

Anova: Single Factor for the PEHA prolinate						
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Column 1	3	0,00517	0,001723333	6,33333E-10		
Column 2	3	0,00491	0,001636667	1,23333E-09		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1,12667E-08	1	1,12667E-08	12,07142857	0,025481	7,708647
Within Groups	3,73333E-09	4	9,33333E-10			
Total	0,000000015	5				

3.2.2. Scaled up equipment for absorption and stripping experiments

On the scaled-up equipment, tests took place both for the absorption and the stripping procedure. The CO_2 absorption and stripping rate was calculated by equation 3.1 (U. E. Aronu, 2009).

$$Q_{CO_2}\left[\frac{mol\ CO_2\ in\ solution}{s}\right] = \left(n_{CO_2}^{in}\left[\frac{mol\ CO_2}{s}\right] - \frac{x_{CO_2}^{out}[mole\ fraction]n_{N_2}\left[\frac{mol\ N_2}{s}\right]}{(1-x_{CO_2}^{out})[mole\ fraction]}\right) \text{(eq. 3.1)}$$

For the stripping rate the n_{CO2}^{in} equals with zero. All the samples were repeated 3 times and the average rate was used for the graphs.

MDEA (25% w/w)

Absorption part

The plots for the MDEA for both absorption and stripping are shown in figure 3.14. The absorption increase follows the same results as the tiny clave assay. That is, the enzyme effect is quite strong and accelerate the absorption rate. For the MDEA solution different amounts of dry enzyme extract were also tested and presented.

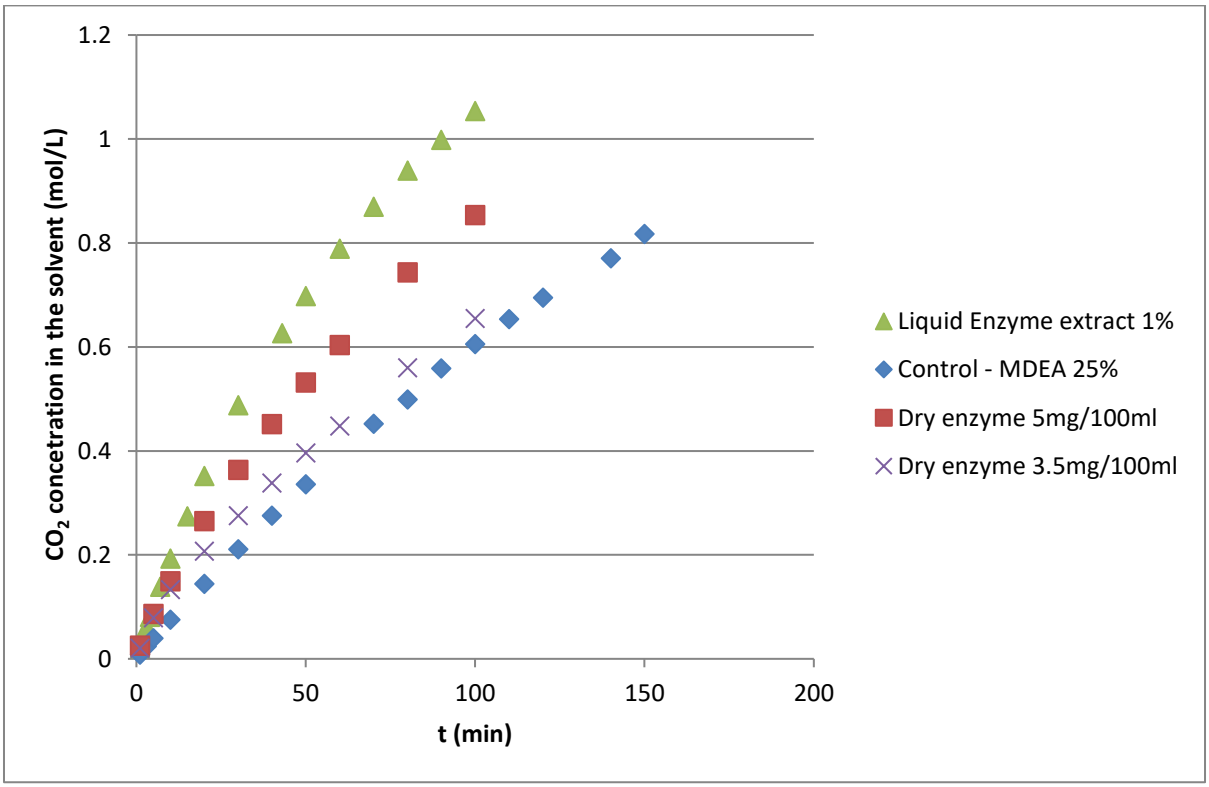


Figure 3.14: CO_2 absorbed in MDEA 25% solution with and without enzyme extract solution

From the experiment's results, it is obvious that the absorption rate is in proportion to the concentration of the lyophilized enzyme in the solution. It is also concluded that the liquid enzyme solution is highly concentrated since it has the highest effect on the absorption rate. Finally, the absorption rate is inversely proportional to the CO_2 concentration in the solution, that is the more CO_2 has been absorbed the more the absorption rate decreases.

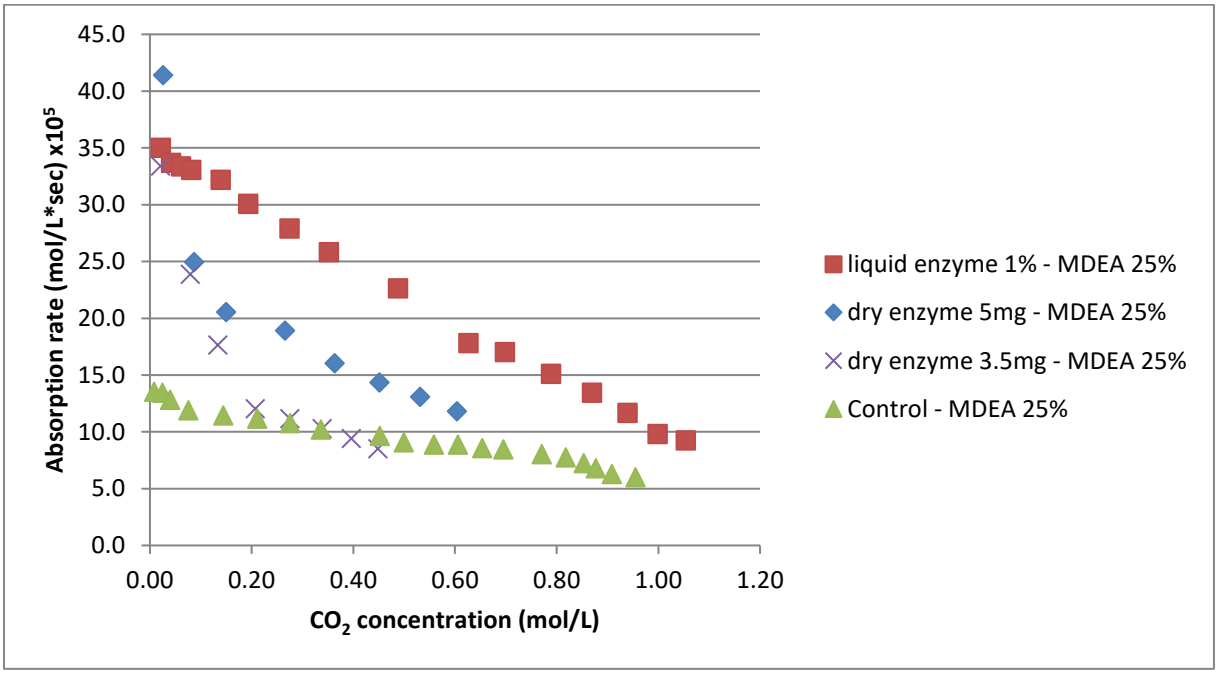


Figure 3.15: CO_2 absorption rite in MDEA 25% solution with and without enzyme

Stripping part

During the stripping part the CO_2 that has been absorbed in the absorption experiment, is now forced out of the solution by using high temperatures that is 80°C. Since the enzyme is quite thermostable it is interesting to research if it also affects the stripping rate.

In figure 3.16 there is a difference in the concentration drop between the solutions with and without enzyme. That could mean that the enzyme presence in the solution affects the stripping rate. However, in figure 3.17 it is obvious that the stripping rates are the same for both the enzyme and the control solution. The main reason for that controversial result could be the fact that during the absorption the solutions were not brought to saturation therefore the start points during the stripping are different.

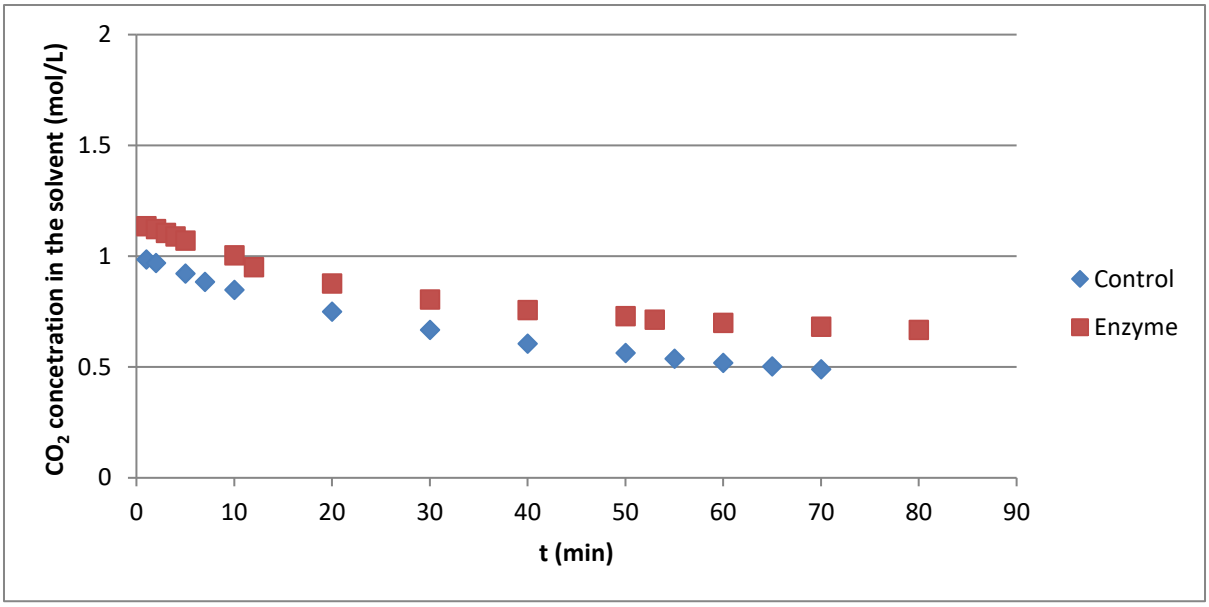


Figure 3.16: Stripped CO_2 in MDEA 25% solution

Based on the results shown in figure 3.17 it can be concluded that the stripping rates do not seem to get affected by the enzyme.

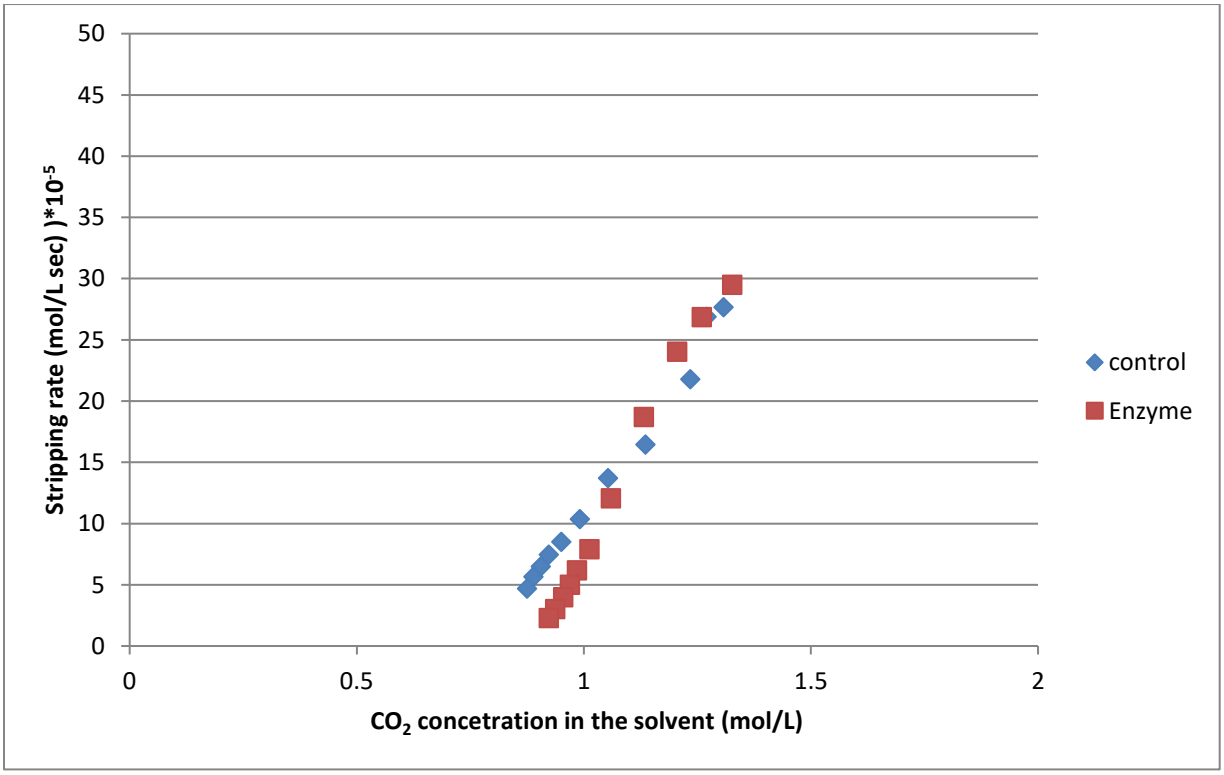


Figure 3.17: CO_2 Stripping rate in MDEA 25% solution

PEHA (25% w/w)

Absorption part

In the tiny clave experiment the pressure drop results for the PEHA solutions showed no difference between the control and the enzyme samples. However, the gravimetrical results showed otherwise proving a higher rate for the sample containing enzyme. Following the results of the tiny clave assay the enzyme in the scale up equipment has a lower impact on the CO_2 absorption in the PEHA solution comparing to MDEA.

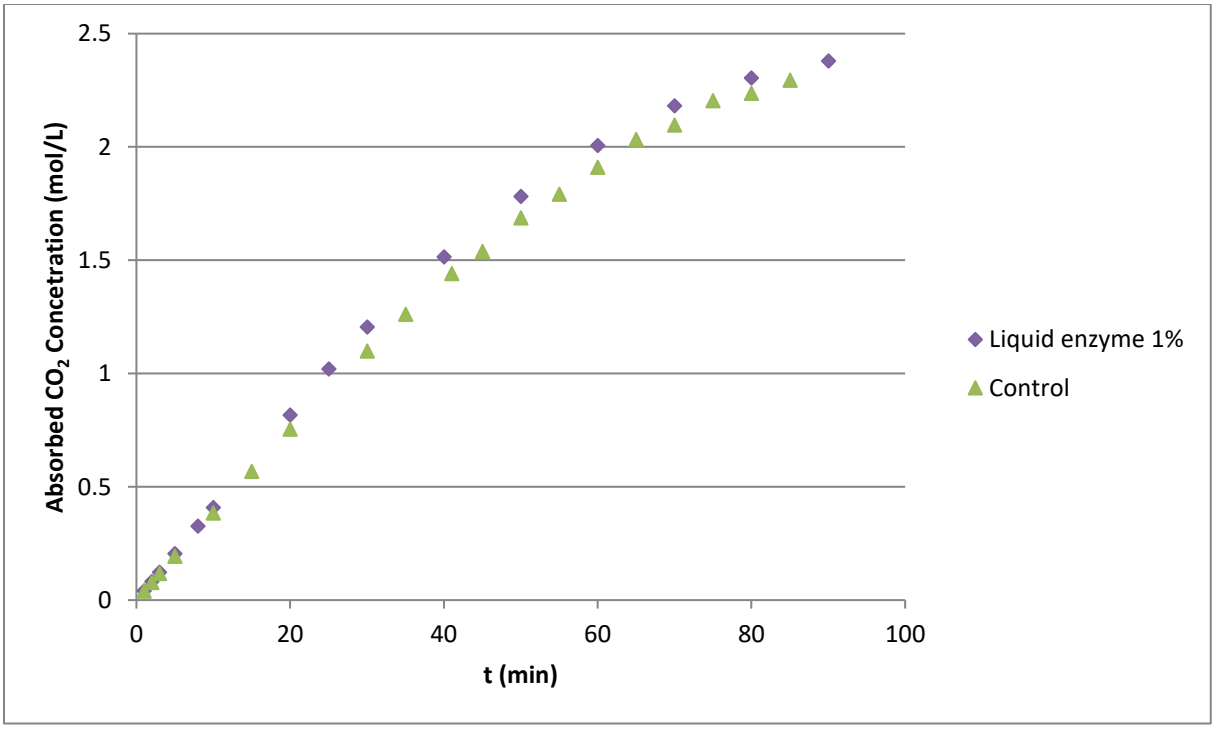


Figure 3.18: CO_2 absorbed in PEHA 25% solution with and without enzyme extract solution

In figure 3.18 the increase of the CO_2 concentration in the PEHA 25% solution seems almost the same for both the enzyme and the control samples, with only light differences for concentrations higher than 1 mol/L. In figure 3.19 watching the rates, the differences become clearer. In this plot the rates of dry enzyme samples are also added to compare.

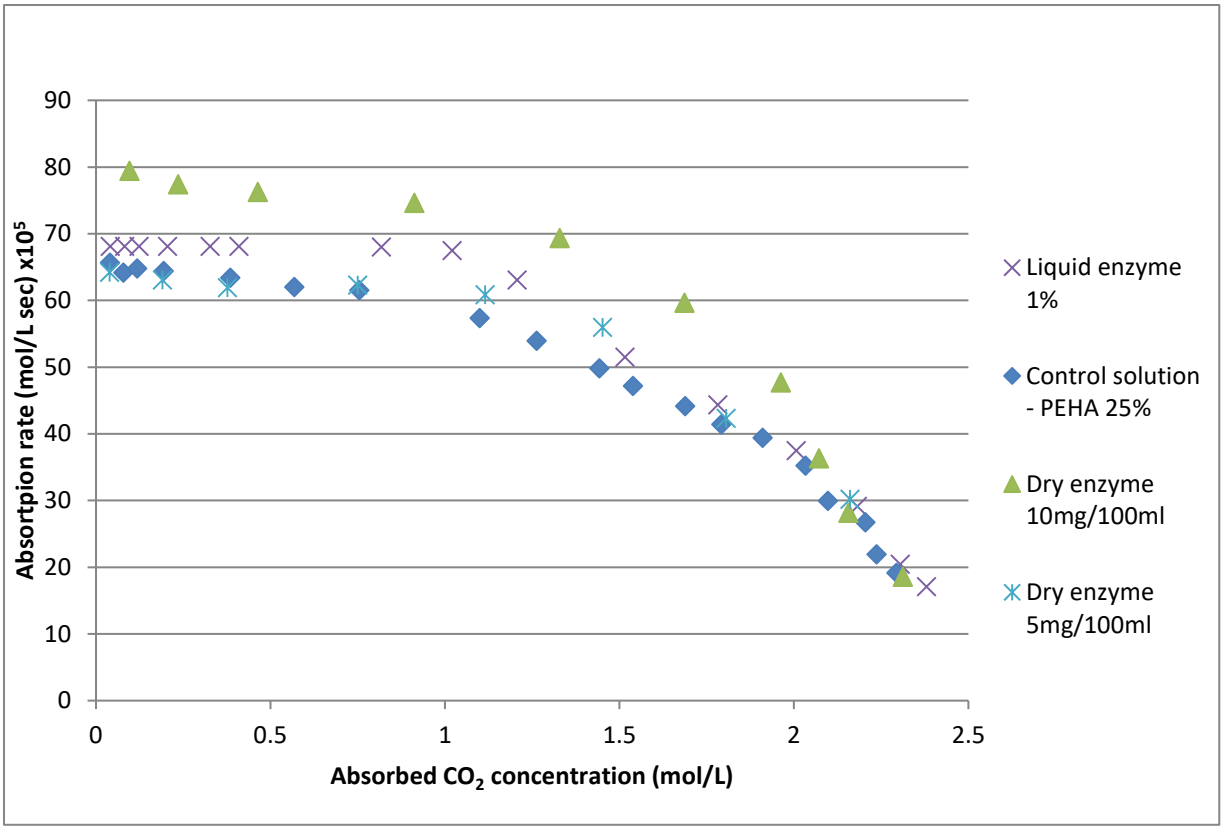


Figure 3.19: CO_2 Absorption rate in PEHA 25% solution with and without enzyme extract

It is obvious that the enzyme has an effect on the absorption rate. The absorption rate is directly connected to the lyophilized enzyme concentration in the solution. The sample of enzyme 50mg/100ml follows the control rate with only exception the part of concentrations from 1 M to 1.5 M. The 1% liquid enzyme extract sample has apparently higher enzyme concentration than 50mg/100ml and lower than 100mg/100ml. It is also observed that all the samples have the same rate when the CO_2 concentration in the solution reaches values higher than 2 M.

Stripping part

Following the absorption experiment, the stripping experiment's results are quite interesting. In figure 3.20, it is shown that since the CO_2 concentration decreases slower in the enzyme solution, the CO_2 is stripped easier control solution. The same results are showed in figure 3.21, where the stripping rates have differences, though the curves tendency seems to be the same for the two samples.

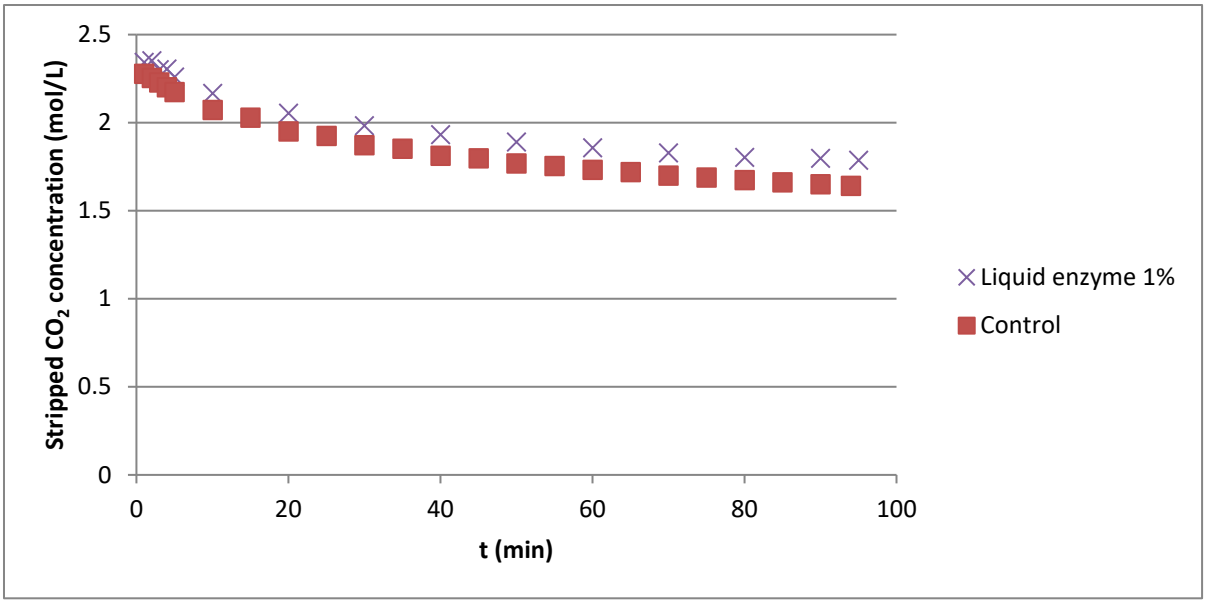


Figure 3.20: CO_2 Stripped in PEHA (25% w/w) solution

A possible explanation to that difference would be that because of the thermostability of the enzyme in 80°C, the enzyme is still active and it reacts to a point with the stripped CO_2 partly resulting the effect of slowing down the stripping procedure.

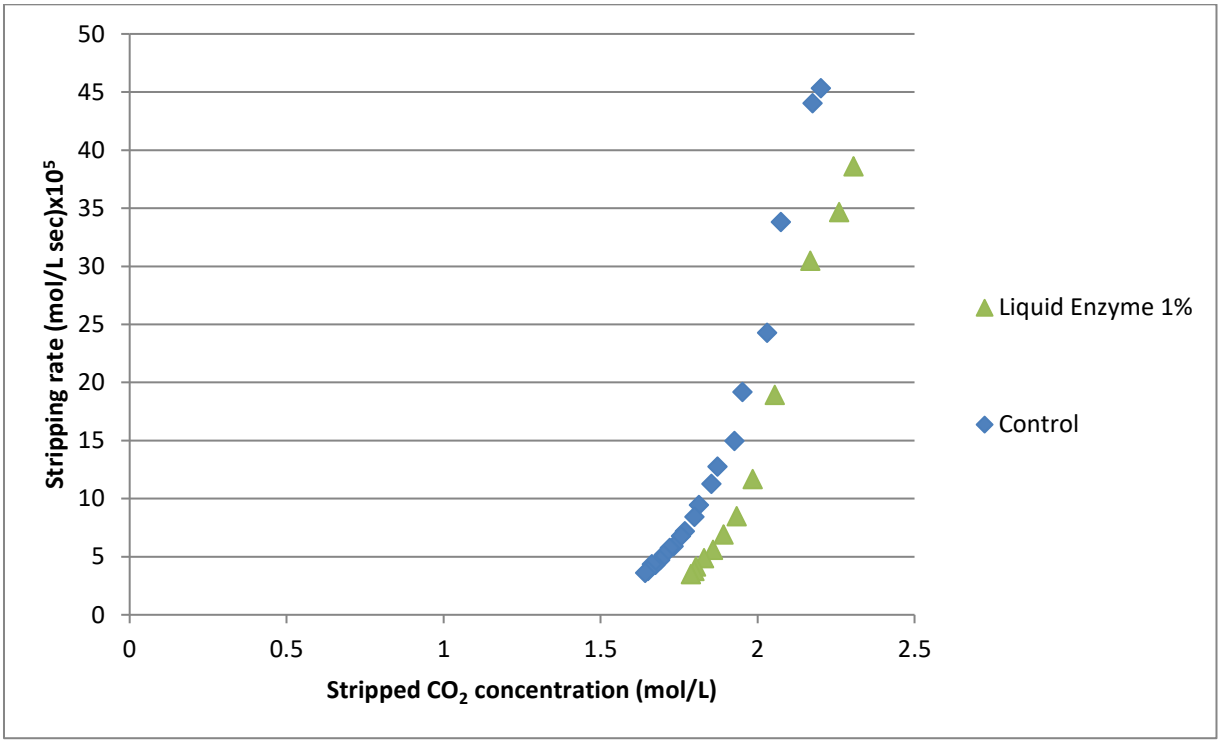


Figure 3.21: CO_2 Stripping rate in PEHA (25% w/w) solution

MEA (6.5% w/w)

Absorption part

In the tiny clave assay the pressure drop plot results showed that the enzyme does not affect the MEA absorption rate. MEA is the most basic amine from the group of the tested amines. Such a basic environment is likely to disactivate the enzyme in small quantities or overlap the effect of the enzyme on the absorption. For that reason, in the experiments on the scale up equipment a solution of MEA 6.5% w/w was chosen in order to observe possible differences between the enzyme and the control samples.

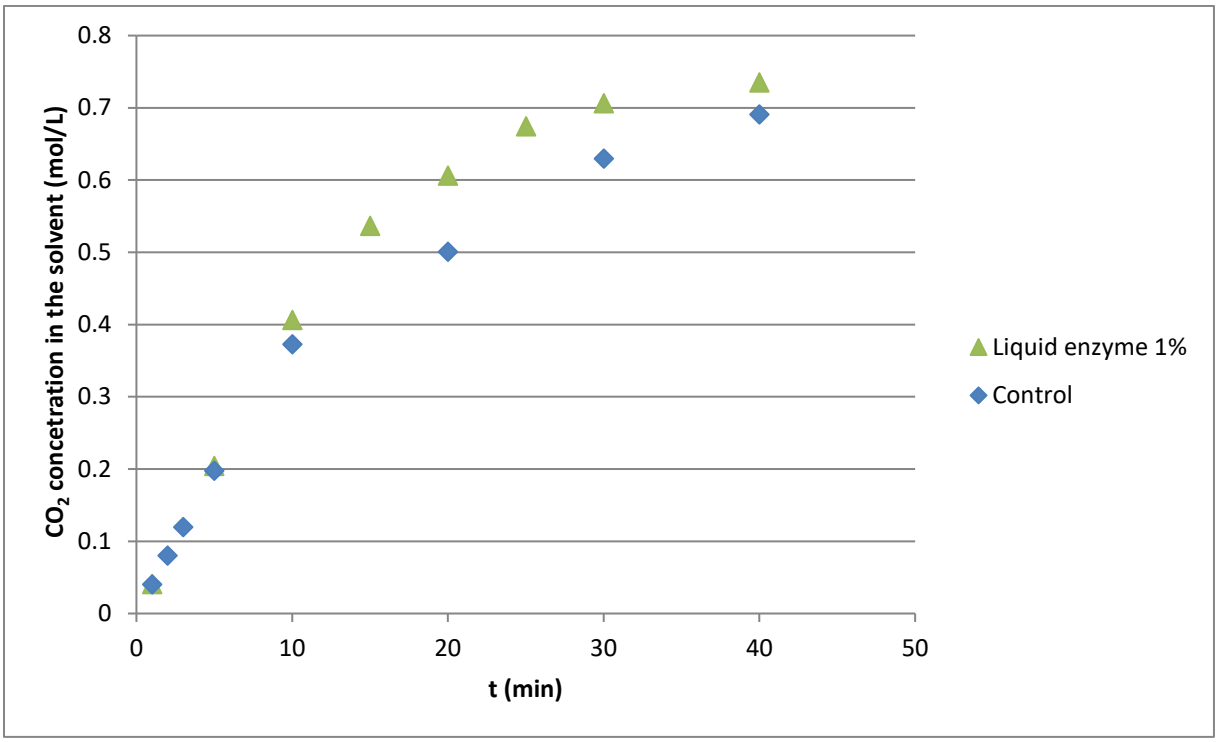


Figure 3.22: Absorbed CO_2 in MEA 6.5% solution with and without enzyme extract solution

The lower concentration MEA, 6.5% w/w, solution provided clearer results about the absorption part. In figure 3.22 it is shown that the absorption rate is higher for the liquid enzyme sample.

In the rate plot as shown in figure 3.23, the absorption rates of dry enzyme samples were also added. In this plot the enzyme effect is clear. Higher enzyme concentrations lead to higher absorption rates. For the dry enzyme sample, it has been observed that although the start rate point is higher than the one in the liquid enzyme sample, the rest of the curve

follows more the control sample rates. In contrast, the liquid enzyme sample maintains a steady rate until the CO_2 concentration reaches 0.4M where the rate starts decreasing.

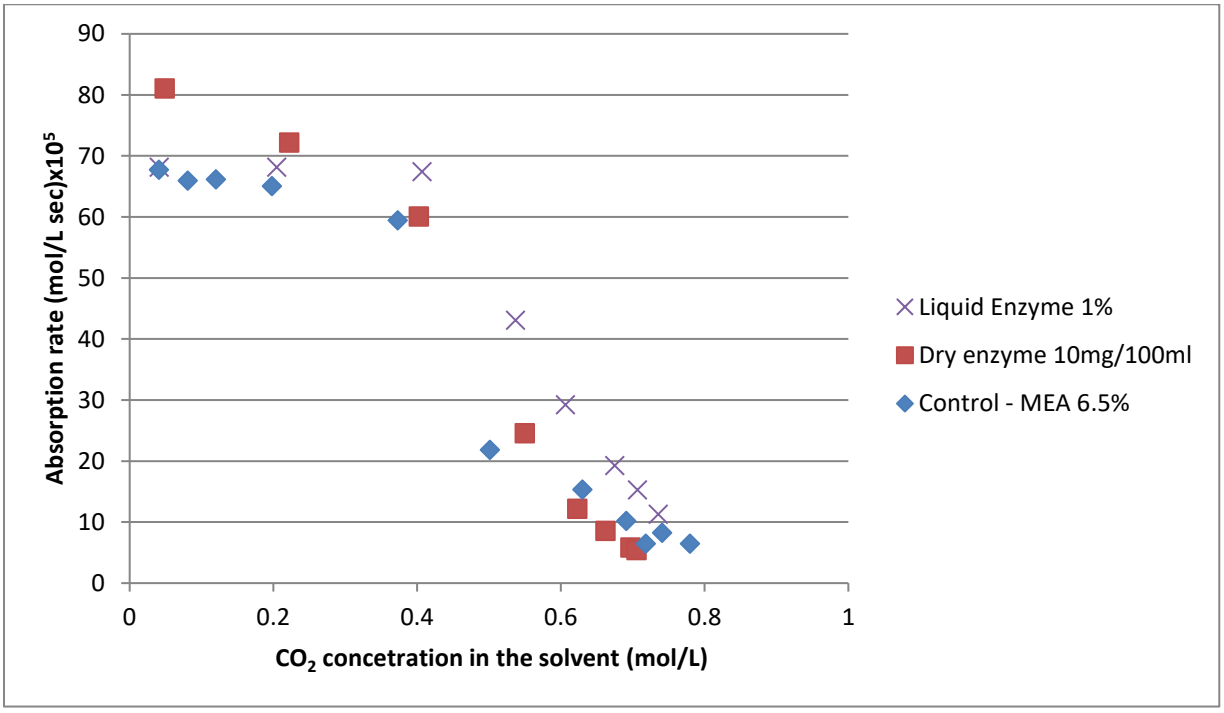


Figure 3.23: CO_2 Absorption rate in MEA 6.5% solution with and without enzyme extract solution

Stripping part

It is observed that the enzyme does not affect the stripping rate as shown in figures 3.24 and 3.25.

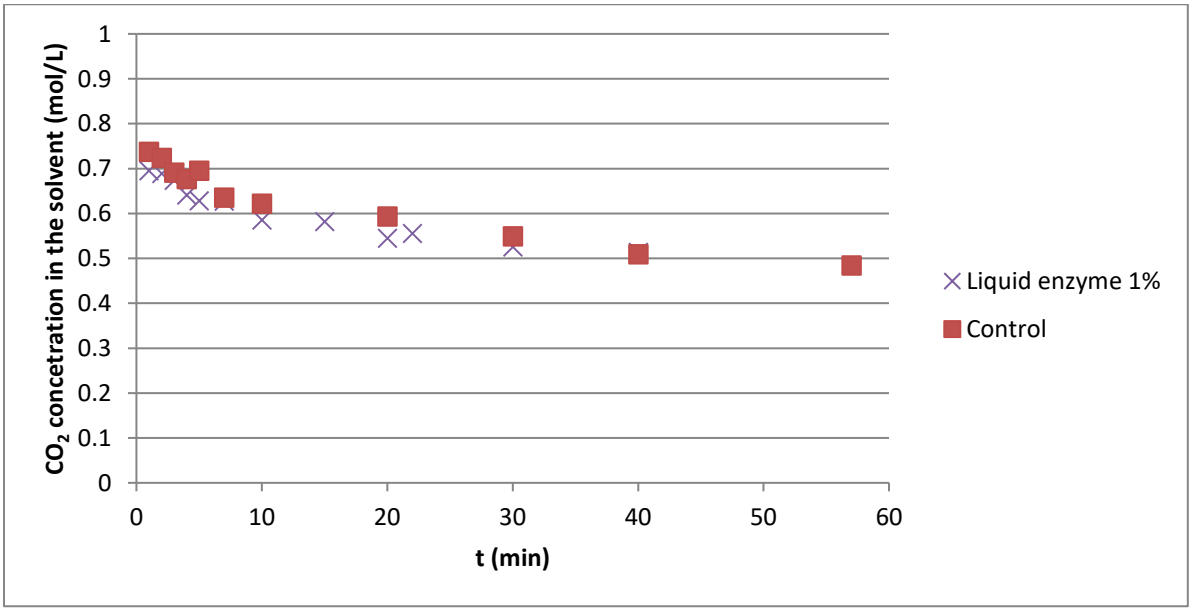


Figure 3.24: Stripped CO_2 in MEA 6,5% solution

It is important to remark that during stripping, there is no enzymatic obstruction observed in the MEA solution as it is likely to happen in the PEHA solution.

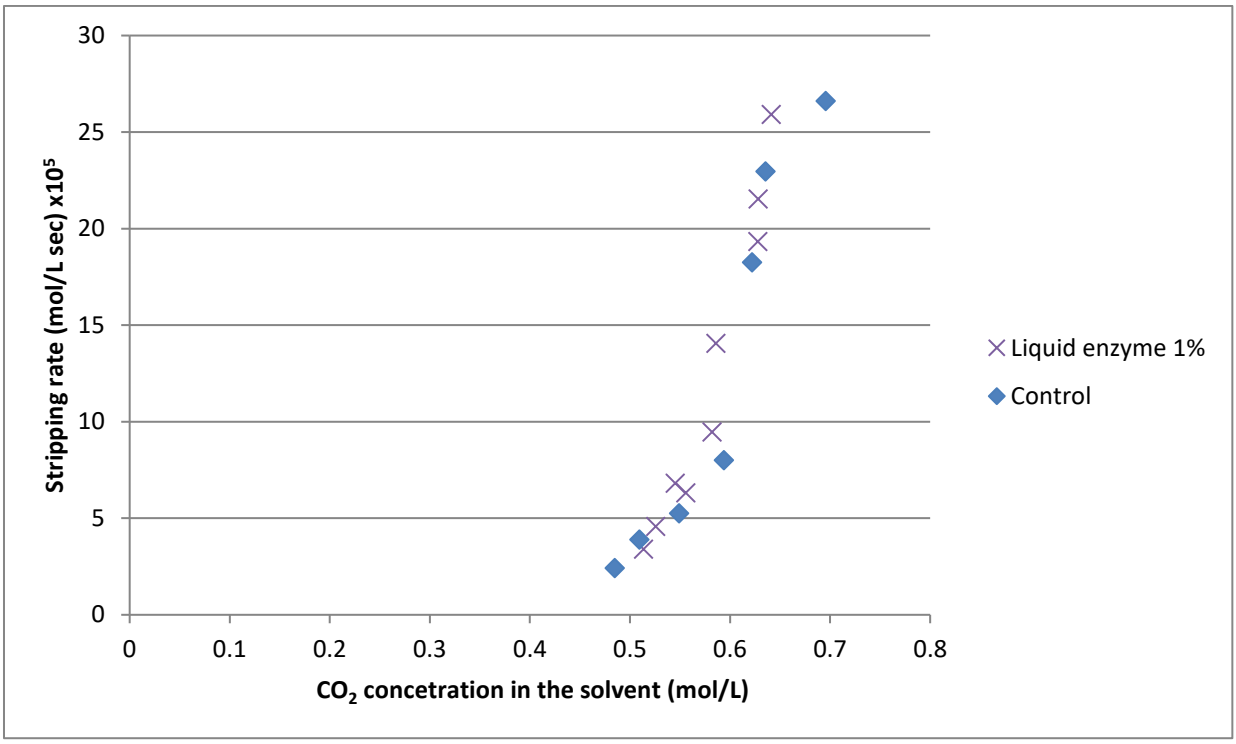


Figure 3.25: CO_2 stripping rate in MEA 6.5% solution with and without enzyme

PEHA - MDEA mix (1:1 ratio)

Absorption part

Coming from the bibliography, the mixtures of different amine solvents tend to have better absorption abilities than each amine alone. In order to test how a mix of amines changes the absorption rates from those of each separately, PEHA and MDEA were mixed in a solution in ratio of 1:1 (12.5% w/w each) to total volume of 100ml. For this experiment samples of liquid enzyme 1% were used.

The results are shown in figure 3.26. The curves of “PEHA enzyme” and “MDEA enzyme” samples were also added in the plot for comparison reasons. It is observed that the mix has significantly higher absorption rate than the MDEA, following the PEHA rate in concentrations lower than 0.5M.

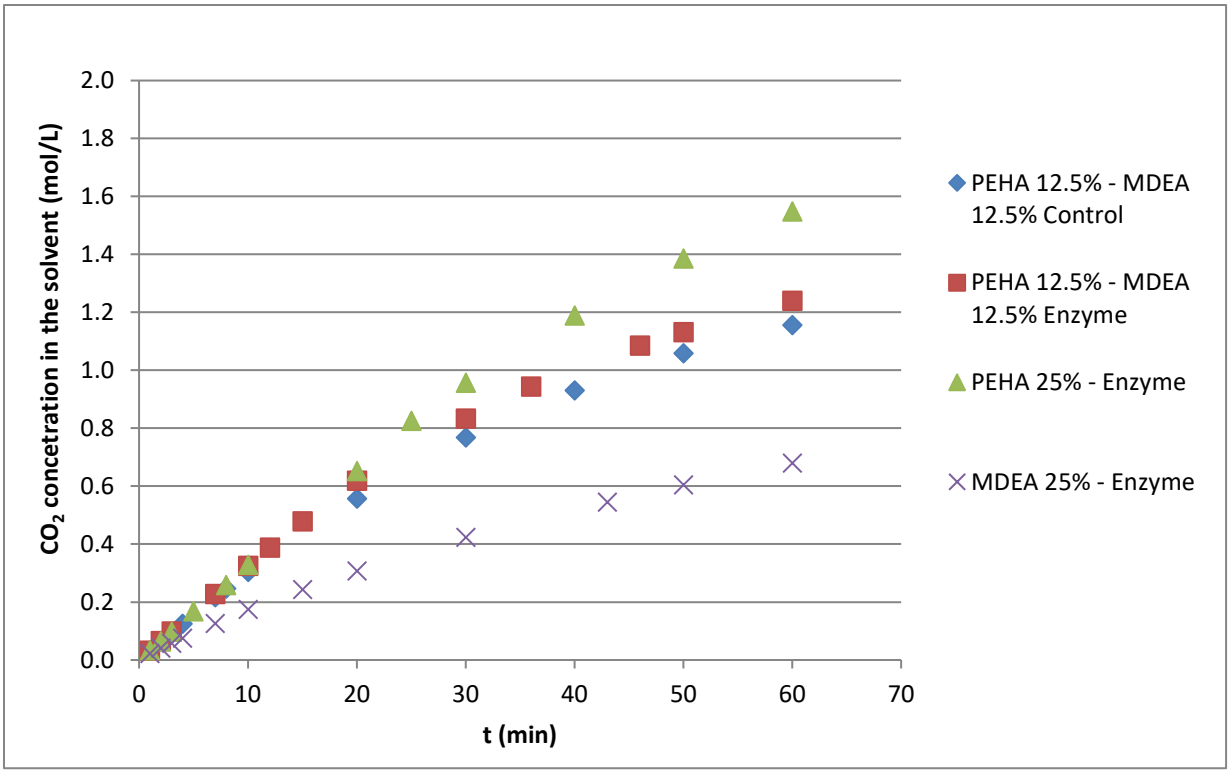


Figure 3.26: CO_2 Absorption in PEHA 12,5% - MDEA 12,5%

The curve tendency follows the PEHA rates and apparently the effect of the enzyme in the mix is quite similar to the PEHA.

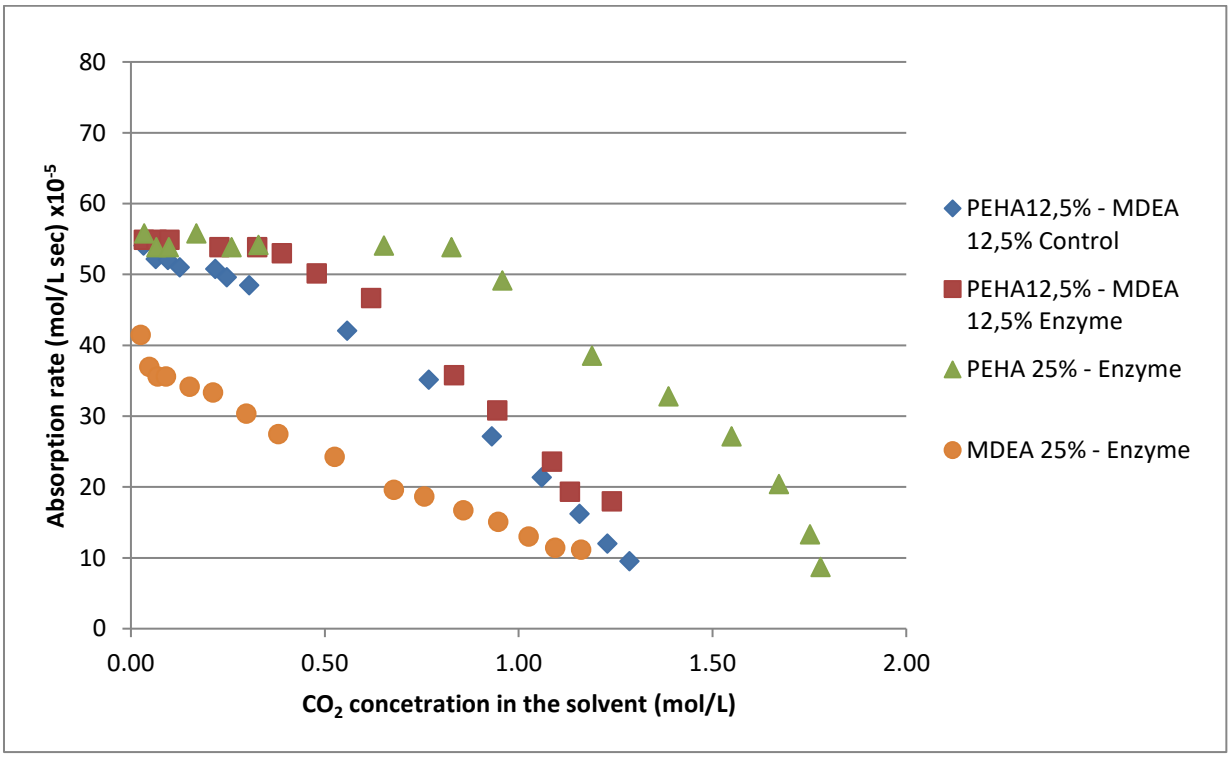


Figure 3.27: CO_2 Absorption rates in PEHA 12,5% - MDEA 12,5%

Stripping part

For the stripping rates a difference is noticed with a higher rate for the control sample. As it was spotted in PEHA experiments and because of the thermostability of the enzyme in 80°C, it is possible during stripping that the enzyme is still quite active and it partly slow down the procedure

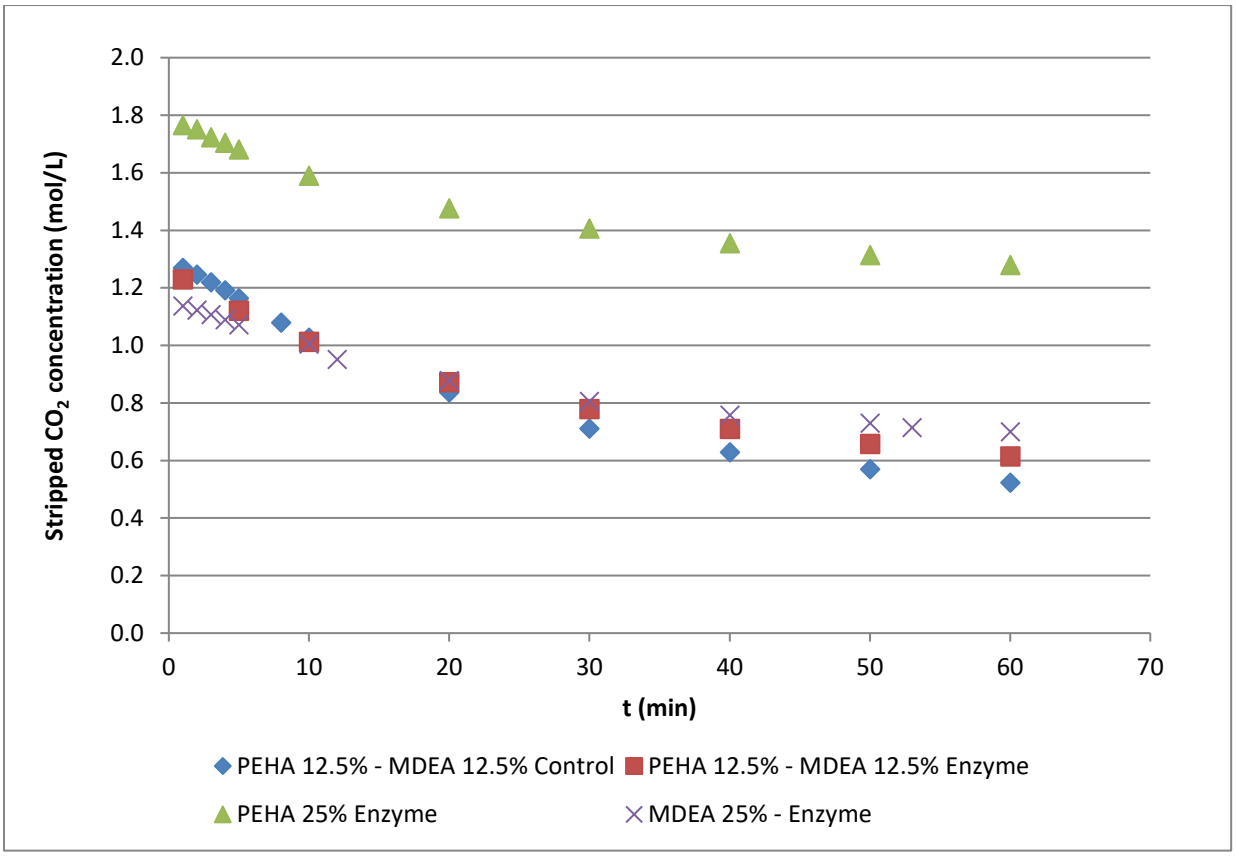


Figure 3.28: CO_2 Stripped in PEHA 12,5% - MDEA 12,5%

At the same time, a difference in the striping rate was observed as the mix has a noticeable higher rate than both PEHA and MDEA when it comes to CO_2 concentration load lower than 1M.

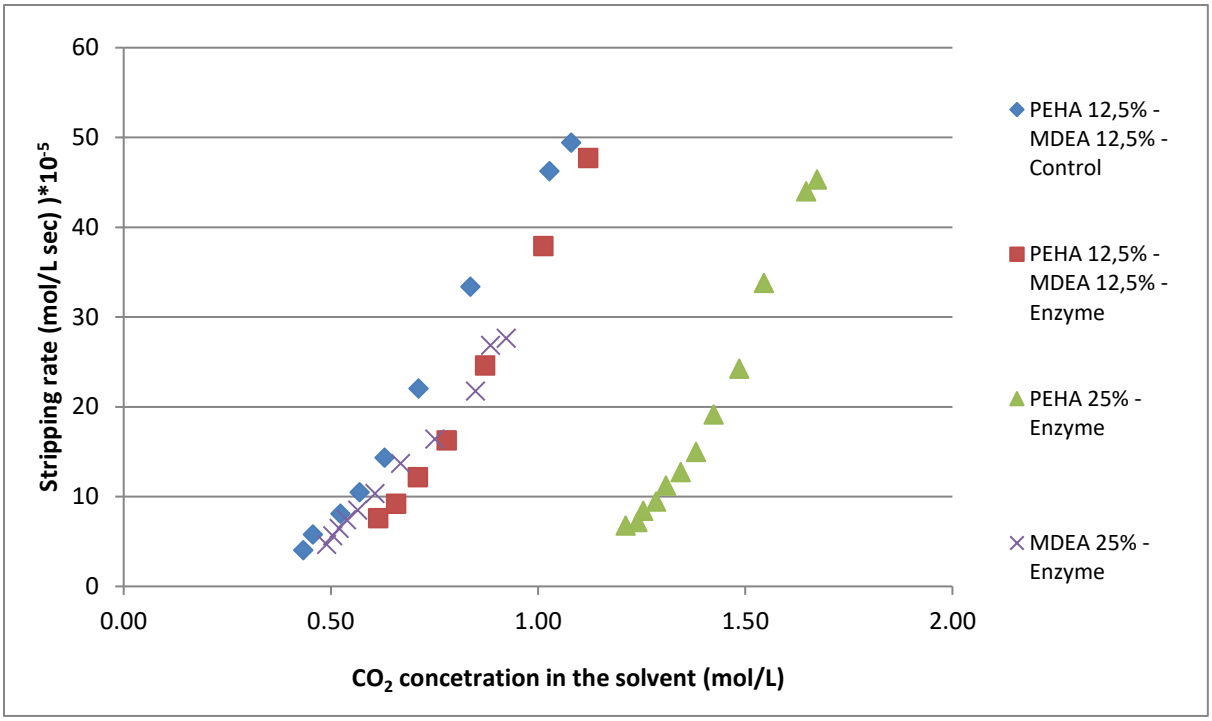


Figure 3.29: CO_2 Stripping rates in PEHA 12,5% - MDEA 12,5%

Comparing the absorption rates for amine solvents, ionic liquids and mixed solutions

It is important to search not only how the enzyme affects the absorption rate of each amine solvent but which amine solvent has the best absorption with and without enzyme, in mixed solutions and in solutions with ionic liquids.

In figure 3.30 the absorption rates of all the tested amines and mixed solutions are presented in one plot. According to that plot the MEA (25% w/w) seems to have the highest absorption rate, holding it at a stable level following by a quick drop for a CO_2 concentration higher than 2 mol/L. The ionic liquid PEHA-prolinate follows with a high absorption rate and a quick drop at 1.5 mol/L. The same state occurs for the MEA (6.5% w/w) following by a quick drop at 0.5 mol/L. In contrast to that, comes the PEHA solution for which the absorption rate follows a slow drop from the start point but it remains quite high through the whole absorption process. The same conditions as the PEHA occur for the mixes “PEHA-MDEA (1:1)” and “PEHA-prolinate - MDEA (1:1)”.

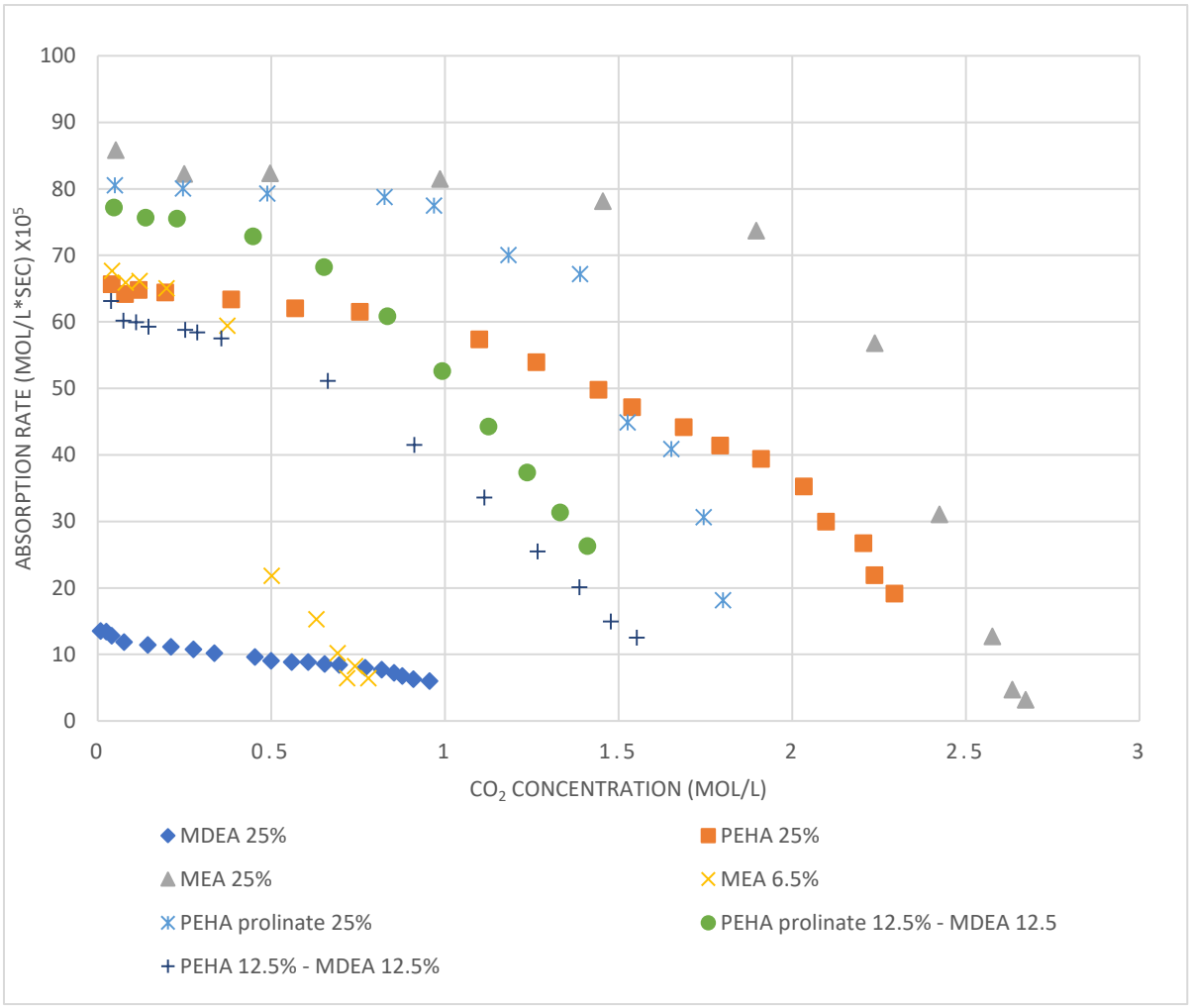


Figure 3.30: Absorption rates of amine solvents (MDEA 25%, PEHA 25%, MEA 25%, MEA 6.5%) and ionic liquids PEHA prollinate 25%

An important increase to the absorption rates occurs with the presence of liquid enzyme solution in the samples. With only exception the MEA (25% w/w) for which the enzyme seems to have no effect on the absorption rates, all the others amine solutions and mixes seem to increase the rate. However, the tendency maintains and the enzyme has the same effect for all the solution. As a result, all the absorption rates have the same increase keeping the PEHA (25% w/w) as the top choice after MEA (25% w/w).

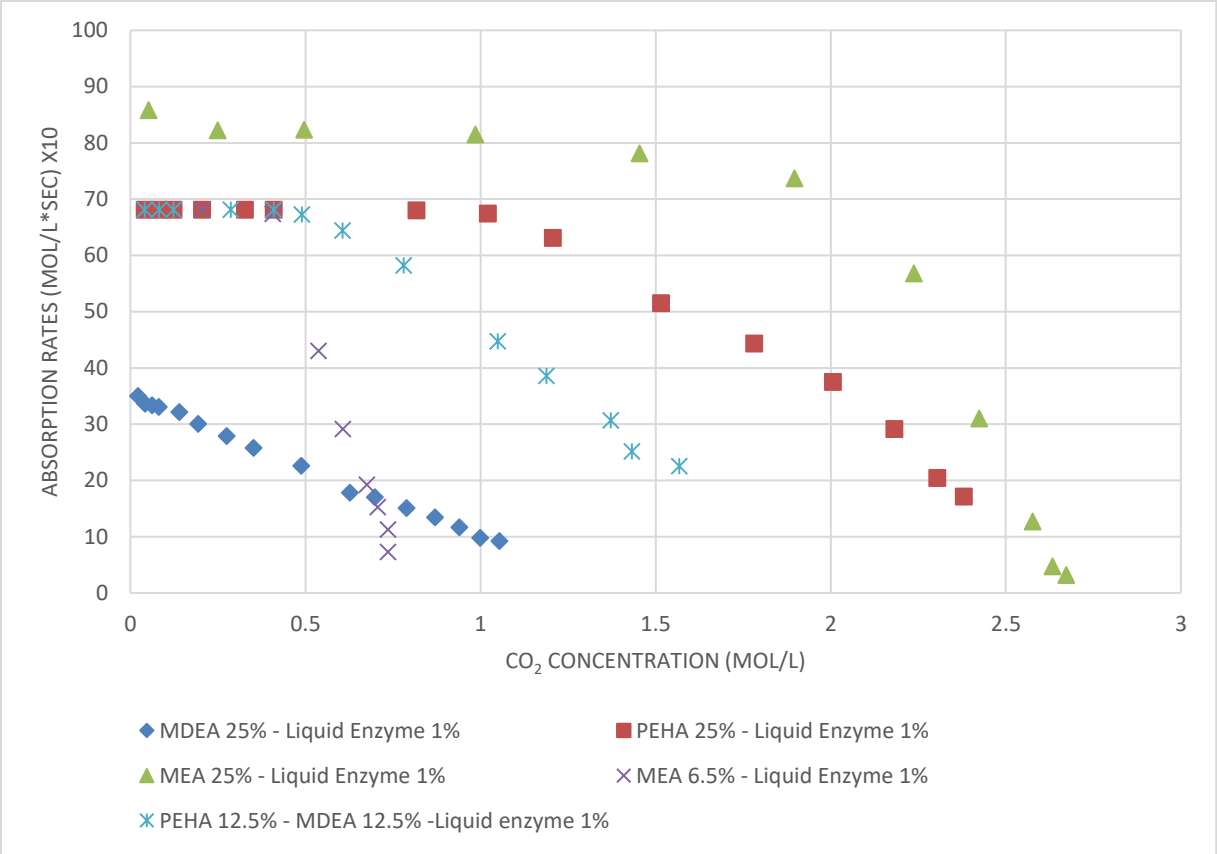


Figure 3.31: Comparing the absorption rates of amine solvents when 1% of the liquid enzyme sample 1% is added to the solution

In figure 3.32 the stripping rates for the amines MDEA, PEHA, MEA (6.5% w/w) with and without liquid enzyme 1% and for the MEA (25% w/w) are presented in one plot. Although the results cannot be compared among amines, it can be concluded that for higher CO_2 concentration in the solution the stripping rate has a higher start point compared to a low CO_2 concentration in the solution.

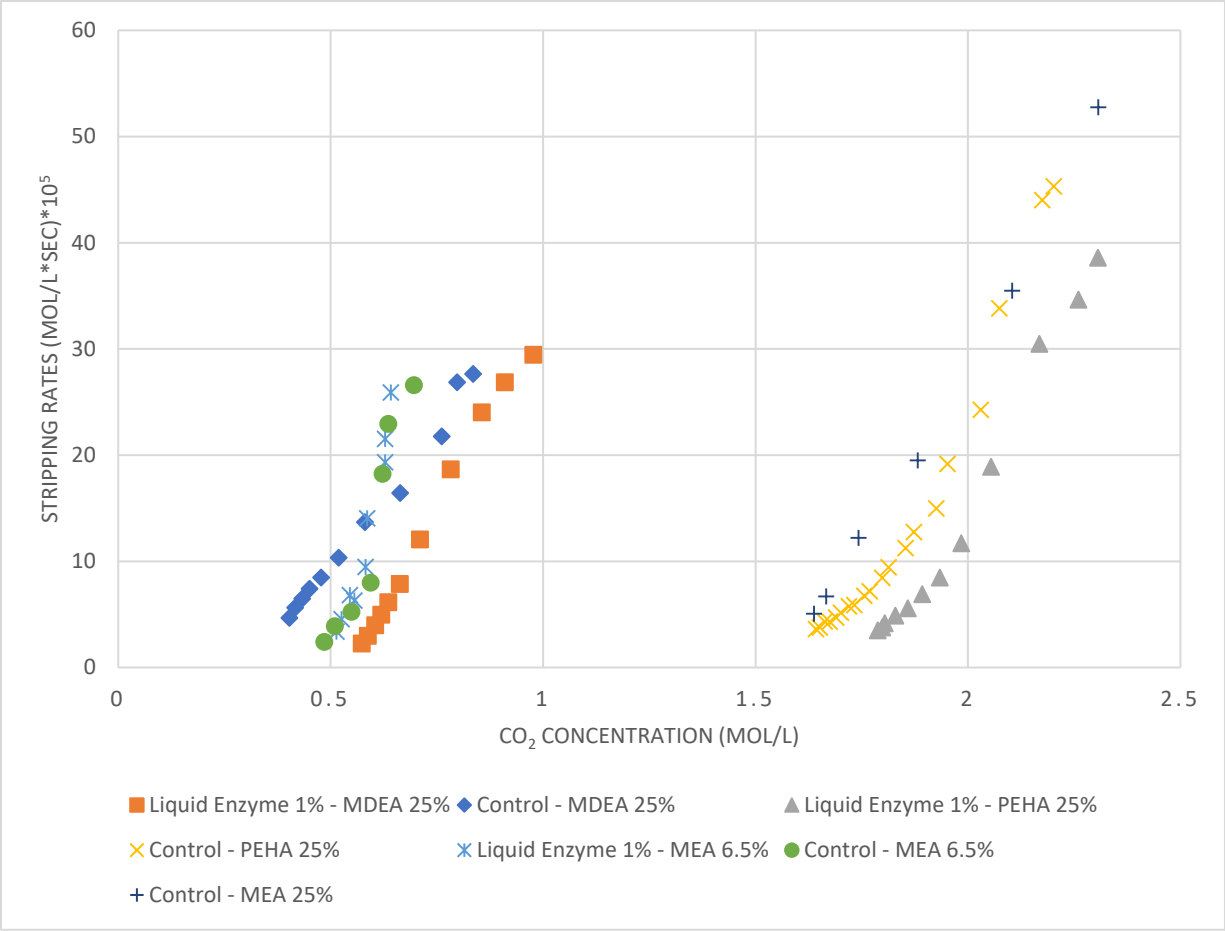


Figure 3.32: Comparing plot of the stripping rates of amine solvents

3.2.3. Tests on scaled-up automated absorption - stripping pilot plant

Absorption experiment on MDEA (25% w/w) solution

The CO_2 absorption in MDEA is shown in figure 3.33. In this equipment the concentration of absorbed CO_2 was measured by the $BaCl_2$ method. According to the plot the pH changes in inverse proportion to the CO_2 concentration. This result was expected since the absorbed CO_2 in the solution reacts to carbonic acid leading to lower pH values.

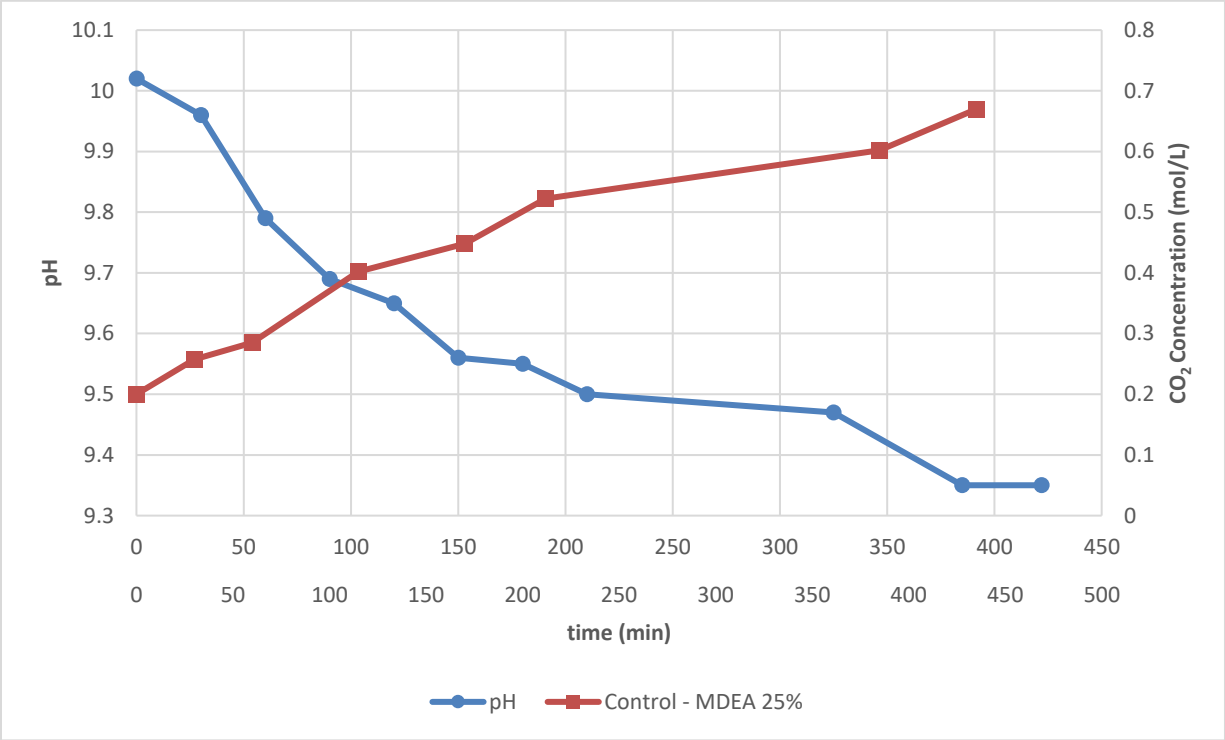


Figure 3.33: CO_2 Absorption rate for MDEA (25% w/w) solution in scaled up pilot plant and the pH drop at 40°C

Stripping part on MDEA (25% w/w)

The values for the CO_2 percentage in the gas outlet given by the CO_2 analyzer were used to measure the CO_2 stripping rate as shown in plot.

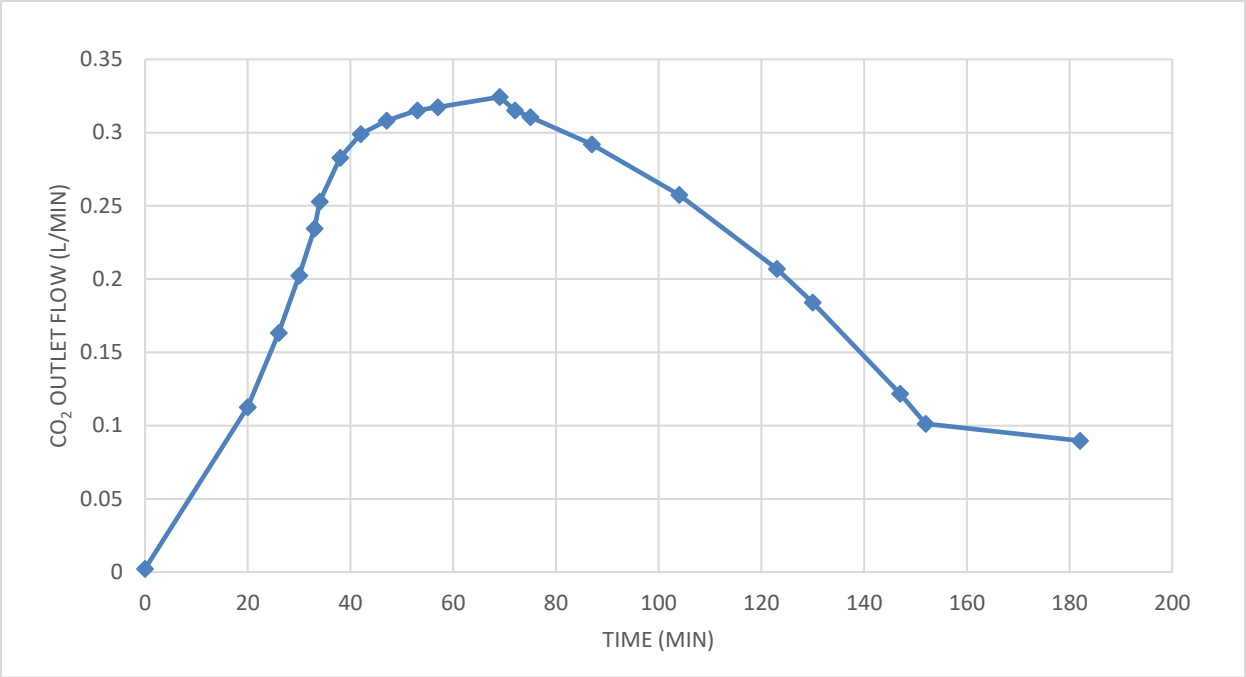


Figure 3.34: CO_2 out flow during stripping for MDEA (25% w/w) solution at 80°C

4.Conclusions

The enzyme exhibits high stability at high temperatures and basic pH environments. By examining the results, it is concluded that the enzyme does act positively for most of the amine solutions that have been tested, significantly increasing the rate of absorption of carbon dioxide and maintaining the desorption rate constant during the CO_2 desorption process. Specifically, by adding an enzyme solution (2% w/w), it has been observed an increase of 40% for MDEA (25% w/w), 9.32% for the PEHA (25% w/w) and 8.01% for MEA (25% w/w). However, there is no change in the capacity of amines in CO_2 by the enzyme's presence in the solution. Among the tested alkanolamines, highest absorption rate was noticed for MEA (25% w/w), followed by the PEHA prolinate ionic liquid. The ionic liquid in a mixture with MDEA (1:1 ratio) shows significant high rate, higher than the rate of MDEA and close to the rate of PEHA. In the future, it is important to further investigate mixtures of amines and ionic liquids and apply them to a pilot plant.

5.Bibliography

A. Bahadori, H. V. (2008). Rapid Prediction of CO2 Solubility in Aqueous Solutions of Diethanolamine and Methyldiethanolamine. *Chemical Engineering Technologies 31*, 245-248.

A. Masohan, M. A. (2009). A simple pH-based method for estimation of CO2 absorbed in alkanolamines. *Indian Journal of Science and Technology*, 59-65.

B. Lv, B. G. (2015). Mechanisms of CO2 Capture into Monoethanolamine Solution with Different CO2 Loading during the Absorption/Desorption Processes. *Environmental science and technology*, 10728-10735.

Byung Hoon Jo, I. G. (2013). Engineered Escherichia coli with Periplasmic Carbonic Anhydrase as a Biocatalyst for CO2 Sequestrtion. *AEM*, 6697-6705.

Cecilia Forsman, G. B.-H. (1988). Production of Active human Carbonic Anhydrase II in E.Coli. *Acta Chemica Scandinavica B 42*, 314-318.

Cheng-Hsiu Yu, C. H. (2012). A review of CO2 capture by absorption and adsorption. *Aerosol and Air Quality research*, 745-769.

D. K. Asimakopoulos, V. I. (2012). *Heat and Mass Transfer.* Athens: Papasotiriou.

Du, M. (2017). Effect of pH on Desorption of CO2 from alkanolamine - Rich Solvents. *American Institute of Physic*.

Evangelista RL, K. A. (1998). Process and economic evaluation of the extraction and purification of recombinant β-glucuronidase from transgenic corn. 607-614.

F. Camacho, S. S. (2008). Kinetics of the reaction of pure CO2 with N-Methyldiethanolamine in Aqueous Solution. *Wiley InterScience*, 204-215.

F. Closmann, G. R. (2011). Degradation of aqueous methyldiethanolamine by temperature and oxygen cycling. *Energy Procedia*, 23-28.

Feron, P. H. (Ed.). (2016). *Absorption-Based Post-combustion Capture of Carbon Dioxide.* Woodhead Publishing.

Ishige T, H. K. (2005). Whole organism biocatalysis. *Curr. Opin. Chem. Biol*, 174-180.

Lars Erik Oi, J. L. (2013). Laboratory rig for atmospheric CO2 absorption and description under pressure. *Energy Procedia*, 1933-1940.

N. Hatcher, C. J. (2014). Predicting corrosion rates in amine and sour water systems: A chemistry-based predictive model predicts corrosion rates in specific processing conditions. *Optimized Gas treating, Inc*, 25-29.

Nathalie J.M.C. Penders-van Elka, P. W. (2012). Kinetics of absorption of carbon dioxide in aqueous MDEA solutions with. *International Journal of Greenhouse Gas Control*, 385-391.

Oscar Alvizoa, L. J. (2014). Directed evolution of an ultrastable carbonic anhydrase for highly efficient carbon capture from flue gas. *PNAS, 111*, 16436-16441.

P.J.G. Huttenhuis, N. A. (2007). Gas solubility of H2S and CO2 in aqueous solutions of N-Methyldiethanolamine. *Journal of Petroleum Science and Engineering 55*, 122-134.

Q. Ye, X. W. (2015). Screening and evaluation of novel biphasic solvents for energy-efficient post-combustion CO2 capture. *International Journal of Greenhouse Gas Control 39*, 205-214.

R.R. Warrior, S. S. (2014). A Modified Assay of carbonic Anhydrase Activity in Tree species. *Biochemistry and Biotechnology Reports 3.1* , 48-55.

S. Santos, J. G. (2016). Scale-up Effects of CO2 Capture by Methyldiethanolamine (MDEA) Solutions in Terms of Loading Capacity. *Technologies*.

Sanoja A Jayarathna, A. W. (n.d.). Dynamic Operations of the Stripping Column of a CO2 Capture Plant. *Telemark Technology Centre*.

U. E. Aronu, H. S. (2009). Solvent selection for carbon dioxide absorption. *Energy Procedia 1*, 1051-1057.

W. L. McCabe, J. S. (6th Edition). *Unit Operations of Chemical Engineers.*

Xiao Luo, S. L. (2017). An improved fast screening method for blended amine-based solvents for post-combustion CO2 Capture. *Energy Procedia 114*, 1848-1854.

Z. Feng, F. C.-G.-T.-T.-M.-B. (2010). Absorption of CO2 in the aqueous solutions of functionalized ionic liquids and MDEA. *Chemical Engineering Journal 160*, 691-697.